A bioinspired scaffold for rapid oxygenation of cell encapsulation systems

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Inadequate oxygenation is a major challenge in cell encapsulation, a therapy which holds potential to treat many diseases including type I diabetes. In such systems, cellular oxygen (O₂) delivery is limited to slow passive diffusion from transplantation sites through the poorly O₂-soluble encapsulating matrix, usually a hydrogel. This constrains the maximum permitted distance between the encapsulated cells and host site to within a few hundred micrometers to ensure cellular function. Inspired by the natural gas-phase tracheal O₂ delivery system of insects, we present herein the design of a biomimetic scaffold featuring internal continuous air channels endowed with 10,000-fold higher O₂ diffusivity than hydrogels. We incorporate the scaffold into a bulk hydrogel containing cells, which facilitates rapid O₂ transport through the whole system to cells several millimeters away from the device-host boundary. A computational model, validated by in vitro analysis, predicts that cells and islets maintain high viability even in a thick (6.6 mm) device. Finally, the therapeutic potential of the device is demonstrated through the correction of diabetes in immunocompetent mice using rat islets for over 6 months.
Cell-based therapies are attractive treatments for a variety of diseases, such as diabetes\textsuperscript{1,2}, liver diseases\textsuperscript{3}, and hemophilia\textsuperscript{4,5}. In particular, the delivery of islets (or stem cell-derived $\beta$-cells) represents a promising therapy for type 1 diabetes (T1D). Islet transplantation via the injection of isolated islets into the liver portal vein or onto the omentum has shown the potential to normalize glycemic control without exogenous insulin in clinical trials, but life-long recipient immunosuppression is required with this procedure\textsuperscript{6,7}.

Cell encapsulation technology offers to protect cells from immune rejection by isolating them from the host using an artificial, semipermeable material, thereby overcoming the need for immunosuppressive agents\textsuperscript{2,8}. Unlike traditional organ transplantations (e.g., pancreas transplantation), wherein the host circulatory system is connected to the transplanted organ via surgical vascular anastomosis\textsuperscript{9}, most islet encapsulation devices (i.e., bioartificial pancreases) remain isolated from the host’s bloodstream after transplantation. Therefore, encapsulated cells are entirely dependent on $O_2$ and other nutrients by passive diffusion from the surrounding blood vessels at the exterior of the device\textsuperscript{10}. It is well documented that $O_2$ is more severely limited than other nutrients because of the relative scarcity of extravascular $O_2$ in vivo\textsuperscript{11}. In the intraperitoneal site, the $O_2$ tension ($pO_2$) is approximately 40 mmHg, and in the subcutaneous site, likely lower\textsuperscript{12}.

A thoroughly investigated approach to improve graft oxygenation is to supply exogeneous $O_2$ in situ. The $\beta$Air device (Beta-O2), for example, supports injections of high concentration $O_2$ into a gas-permeable chamber adjacent to hydrogel-encapsulated cells\textsuperscript{13,14}. Another strategy is the local production of $O_2$ using chemical reactions\textsuperscript{15,16} or electrolysis\textsuperscript{17,18}. Though these strategies have all demonstrated the benefit of adequate $O_2$ supply to encapsulated cells, remaining limitations include increased device complexity and the requirement of patient compliance to maintain $O_2$ provision. $O_2$ transport in hydrogels is invariably dependent on its permeability, the product of the solubility and diffusivity coefficients, both of which are low in aqueous media such as hydrogels and tissue. An alternative, possibly simpler or complementary approach is thus to improve the $O_2$ permeability of the encapsulating material.

In the absence of supplemental $O_2$ provision, theoretical analyses suggest that islets should be within a few hundred micrometers from the bloodstream in surrounding tissue to avoid hypoxia\textsuperscript{19,20}. Based on this design principle, the cell module of an encapsulation system should be exceedingly thin to support favorable oxygenation\textsuperscript{12,21}. For spherical microcapsules which are endowed with a high surface area to volume ratio, a diameter of $\sim$1000 μm is widely used\textsuperscript{11,22,23}. Similarly, cylindrical cell-laden hydrogel fibers are commonly designed from 350–1000 μm in diameter\textsuperscript{24–26}, and planar slabs (the geometry endowed with the lowest surface area to volume ratio), typically from 250–600 μm in thickness\textsuperscript{8,13,14}.

Islet cell transplantsations require approximately 500 k islet equivalent (IEQ) of human islets (5–10 k IEQ per kg body weight) to reverse diabetes\textsuperscript{3}, and cellular treatments for liver diseases and hemophilia require similar cell volumes\textsuperscript{8}. Because $O_2$ diffusion limitations restrict hydrogel thickness, devices can only be scaled along one or two spatial dimensions to accommodate this requisite cell payload, and thus an unreasonably large estimated device length, surface area, or number is required. It is estimated that meters of a cylindrical fiber, hundreds of square centimeters of a planar slab, or $\sim$100,000 microcapsules are needed to deliver a curative islet dose. Increasing the system’s $O_2$ permeability would allow devices to be scaled in three dimensions, facilitating the design of reasonable and surgically convenient device geometries (i.e., shorter in length, smaller in surface area, or lower in number).

The physiology of insects presents a creative solution to rapid $O_2$ distribution across multi-millimeter scales. Instead of using circulatory blood for tissue oxygenation as in vertebrates\textsuperscript{27}, insects transport gaseous oxygen via a gas-filled channel network, known as the tracheal system, which is distributed throughout their bodies; this system is also present in some aquatic insects without spiracles\textsuperscript{28,29}. Inspired by this clever mechanism of rapid gas-phase $O_2$ distribution, we pursued the design of an air-filled scaffold for islet encapsulation to overcome the thickness limitation for $O_2$ diffusion, which we name SONIC (Speedy Oxygenation Network For Islet Constructs) (Fig. 1).

We highlight the bio-inspiration by analyzing the tracheal anatomy of a larva of the mealworm beetle (Tenebrio molitor) (Fig. 1a), which guided the design of the SONIC scaffold. High-resolution X-ray computer tomography (Nano-CT) scanning was used to visualize the tracheal network in the mealworm (Supplementary Fig. 1), showing its thorough distribution throughout the body in a ladder-like geometry\textsuperscript{30,31} (Fig. 1b). It is evident from this pattern that $O_2$ is delivered to insect tissue through the air channels in the trachea (Fig. 1c). Importantly, the $O_2$ diffusion coefficient in the gaseous phase is around 10$^4$ times higher than that in water or biological tissue\textsuperscript{29,32}. Thus, the tracheal network leverages rapid gas-phase $O_2$ transport, allowing insects to efficiently distribute $O_2$ throughout their bodies\textsuperscript{33}. This innovative $O_2$ transport mechanism allows some insects to grow to incredibly large sizes\textsuperscript{34}, such as the Goliath beetle larvae reaching the size of a mouse, the Dynastes Hercules larvae reaching 147 mm long with a weight of 144 g, and the Megasoma larvae reaching a maximum dorsal width of 225 mm\textsuperscript{35}.

Herein, the incorporation of the SONIC scaffold in a hydrogel-based cell encapsulation system intentionally recapitulates the efficiency of rapid $O_2$ transport of the insect tracheal network, oxygenating deeply encapsulated cells within thick devices (Fig. 1d). We confirm the rapid and penetrating $O_2$ transport capability through the SONIC scaffold visually using an $O_2$ imager. Additionally, we demonstrate that the SONIC device can maintain high cell viability and robust function of rat islets in immunocompetent diabetic mice for over 6 months. The bio-mimetic SONIC cell delivery system solves the problem of slow and non-penetrating $O_2$ transport in thick bulk hydrogels.

**Results**

**Design and fabrication of the SONIC device.** To develop a SONIC scaffold suitable for cell encapsulation and supporting of rapid $O_2$ transport, two features, difficult to achieve simultaneously, were essential: (1) an internal microstructure that was bicontinuous and non-wettable to provide unobstructed microchannels for $O_2$ flow similar to the insect trachea and (2) an external surface that was wettable to allow cell-laden hydrogel precursor to infiltrate. We conjectured that a hydrophobic material for the scaffold skeleton would resist water infiltration. We designed a two-step process, forming first a superhydrophobic (i.e., low surface energy and high roughness) scaffold skeleton with continuous internal pores and then an adherent hydrophilic coating only on the external surface.

Accordingly, poly(vinylidene fluoride-co-hexafluoropropylene) (PVDF-HFP) (Fig. 1e) was selected as the skeletal material, which imparts hydrophobicity via the highly fluorinated -CF$_3$ group of the HFP unit\textsuperscript{36}, while providing high solubility in polar solvents via the unique polarity of the alternating -CH$_2$- and -CF$_2$- groups of the VDF unit along the polymer chain\textsuperscript{37}. PVDF-HFP solution could then be subjected to an immersion
precipitation process to produce the interconnected porous and rough microarchitecture via phase separation. Furthermore, PVDF-HFP is resistant to hydrolytic, oxidative, and enzymatic breakdown, and is therefore advantageous for use in vivo.

The SONIC scaffold was fabricated as shown in Fig. 1f. A multilayer poly(lactic acid) (PLA) mold (Supplementary Fig. 2), complementary to the targeted ladder network geometry (Fig. 1d), was first 3D printed. Subsequently, a PVDF-HFP solution (15 wt % in acetone) was filled into the PLA mold and immersed in a water/ethanol (v/v = 1/1) bath for the phase separation process yielding the internal porous microstructure containing polymer-rich regions and polymer-poor regions (i.e., the air channels). Finally, the ladder-like PVDF-HFP scaffold was isolated after selective extraction of the PLA mold using chloroform (Fig. 1g and Supplementary Movie 1).

The structural analysis confirmed that the desired macro- and microarchitecture (Fig. 1h) of the SONIC scaffold was achieved. Nano-CT imaging illustrated an internal microporous structure with a pore size of a few micrometers (Fig. 1i). Additionally, renderings of 3D reconstruction images revealed that the internal air channels were

Fig. 1 Design and fabrication of the biomimetic SONIC scaffold. a A digital image of a larva of the mealworm beetle (*Tenebrio molitor*). b 3D reconstruction of Nano-CT images of the tail of a mealworm (left) and the segmented gas-phase tracheal system (right) inside the body. c Schematic illustrating the tracheal system in a mealworm and O₂ delivery to the surrounding cellular tissue through the tracheae. d A schematic illustrating O₂ delivery from the transplantation site into the cell encapsulation system through a tracheal ladder network-like SONIC scaffold. e Chemical structure of the fluoropolymer PVDF-HFP. f Fabrication of the ladder-like SONIC scaffold. g A digital image of the SONIC scaffold. h Schematics representing the macro- and microarchitecture structure of the SONIC scaffold. i A Nano-CT image of the porous scaffold (the asterisks indicate the pore regions). j 3D reconstruction of Nano-CT images of a selected region (26 × 34.84 × 5.36 µm) inside the SONIC scaffold showing the bicontinuous microstructure (the diffuse red coloring indicates the air phase in the porous skeleton). k Skeletal networks for the polymeric (blue) and the porous (red) regions of the SONIC scaffold.
Fig. 2 Fabrication and characterizations of the SONIC device. a Schematic illustration of the device fabrication (side view). b, c False-colored SEM images of the SONIC scaffold (b) and polydopamine-coated SONIC scaffold (c). One representative of 3 independent experiments is shown. d, e Digital images of water droplets (colored with food dye) and contact angle goniometer-captured images of a water droplet on a rectangular prism SONIC scaffold before (d) and after (e) polydopamine modification. f A false-colored SEM image of the polydopamine-coated SONIC scaffold with deposited CaSO₄ crystals. One representative of 3 independent experiments is shown. g Stereo microscope image of the SONIC device (top view). h SEM/EDS elemental mapping of F, N, and S on a polydopamine-coated SONIC scaffold with deposited CaSO₄ crystals. The red arrows indicate the lack of polydopamine at a coating crack location. One representative of 3 independent experiments is shown. i SEM image of the polydopamine-coated SONIC scaffold and the corresponding element N distribution profile across a polydopamine coating crack. One representative of 3 independent experiments is shown. j SEM image of the cross-sectional polydopamine-modified SONIC scaffold, showing no polydopamine inside the scaffold. One representative of 3 independent experiments is shown. k An H&E staining slice of an islet encapsulation device showing the polydopamine located at the interface between the SONIC scaffold and alginate hydrogel. One representative of 10 replicates is shown. l Captured images during the perfusion test using a cylindrical SONIC scaffold. A pump-connected needle was inserted into one end of a cylindrical SONIC scaffold, and the other end of the SONIC scaffold was immersed into a vial containing water phase (top, colored with green food dye) and chloroform phase (bottom, colored by Nile Red dye). m Schematics representing the distribution of water and chloroform during the perfusion test.

Indeed continuous (Fig. 1j, Supplementary Fig. 3, and Supplementary Movie 2). Furthermore, skeletal network analysis for the polymeric skeleton and the air channels confirmed the bicontinuous structure (Fig. 1k). These characterizations confirmed that the SONIC scaffold recapitulated the essential features of the tracheal system necessary for rapid gas-phase O₂ transport.

To integrate the SONIC scaffold into a hydrogel-based cell encapsulation device (Fig. 2a), it was critical to render the scaffold surface wettable. Dopamine can self-polymerize to form a surface-adherent hydrophilic polydopamine film onto a wide range of substrates, including on hydrophobic surfaces. Thus, we applied the mussel-inspired hydrophilic polydopamine coating on the scaffold’s surface (Fig. 2b, c and Supplementary Fig. 4) but not the internal pores. To test the efficacy of this process, water contact angle (CA) measurements were performed to characterize the wettability of the SONIC scaffold before and after polydopamine modification. The CA of polydopamine-coated SONIC scaffold decreased from the original 151° (Fig. 2d and Supplementary Movie 3) to 44° (Fig. 2e), showing the substantially increased wettability.

Following surface hydrophilicity modification, a cell-laden hydrogel was applied via a simple in situ procedure. CaSO₄ was deposited onto the SONIC scaffold surface as a crosslinker source (Fig. 2f) for the following application of a cell-containing alginate solution (SLG 100, 2 wt%). In comparison with calcium salt (CaCl₂), which is more commonly used for alginate cross-linking, CaSO₄ has a much lower solubility, retarding the gelation rate to provide time for the alginate solution to penetrate into the scaffold skeleton before gelling (Fig. 2g). Furthermore, the slow dissociation of Ca²⁺ from the CaSO₄ crystals produces a more uniform and mechanically stronger alginate hydrogel than fast gelation in a CaCl₂ bath.

Scanning electron microscopy (SEM)/energy-dispersive X-ray spectroscopy (EDS) mapping was used to analyze the component...
distribution on the SONIC scaffold. Fluorine (F), nitrogen (N), and sulfur (S) were chosen as the specific elements to identify PVDF-HFP, polydopamine (PDA), and CaSO₄, respectively (Fig. 2h). The SEM/EDS line profile of the N signal across a coating crack showed the lack of polydopamine inside the scaffold (Fig. 2l). In addition, an SEM cross-sectional image of the polydopamine-modified scaffold showed that the polydopamine coating was only distributed on the surface (Fig. 2j). Furthermore, hematoxylin and eosin (H&E) staining of a slice of an islet encapsulation device also confirmed that the polydopamine was only located at the interface between the scaffold and alginate hydrogel (Fig. 2k). These characterizations confirmed that hydrophilicity modification was limited to the scaffold surface and therefore unlikely to affect the hydrophobicity of internal regions of the scaffold.

We performed a perfusion study to test the ability of the SONIC scaffold to prevent water from wicking into the internal pores. A cylindrical scaffold was inserted into a vial containing a water phase and a chloroform phase (Fig. 2l). The chloroform quickly penetrated the scaffold by capillary action and spread throughout the whole scaffold by suction force applied on the top, whereas the water was excluded and remained outside the scaffold even after all chloroform was withdrawn (Fig. 2m). The flow of chloroform throughout the scaffold also confirmed the SONIC scaffold’s bicontinuous porous structure, validating visual observations in previous Nano-CT scanning characterizations (Fig. 1j, k).

**Rapid O₂ transport through the SONIC scaffold.** O₂ distribution mapping was performed on an electron paramagnetic resonance (EPR) imager to characterize O₂ transport through the SONIC scaffold (Fig. 3). Briefly, a SONIC scaffold or a control PLA filament was inserted in 1% gelatin containing EPR spin probe (OX063-d24, 1 mM) in a glass tube (Fig. 3a–c). First, the system was deoxygenated using N₂ to reduce Pₐ close to 0 mmHg. After deoxygenation, the system was exposed to a gas mixture containing a Pₐ of 40 mmHg, and the Pₐ change in gelatin was continuously monitored until a steady-state (40 mmHg) was approached. The average Pₐ in gelatin with the PLA control insert slowly climbed to ~35 mmHg after 11 h (Fig. 3d). Spatial Pₐ distribution plots showed a gradient descending from top to bottom in the tangential plane and uniform profiles in the transverse plane (Fig. 3e). On the other hand, the average Pₐ in gelatin with the SONIC scaffold rapidly increased to the equilibrium level of 40 mmHg within ~2 h (Fig. 3f). Moreover, spatial Pₐ images in the transverse plane showed a radial gradient emanating from the location of the SONIC scaffold before achieving steady-state, and profiles in the tangential plane revealed high Pₐ levels at the bottom of the sample near the SONIC scaffold, even in the first image collected after exposure to the gas mixture (Fig. 3g).

A computational model, developed to simulate spatial O₂ transfer over time in this system (Supplementary Fig. 5), was in general agreement with experimental results. In silico, the SONIC scaffold distributed O₂ more efficiently through the gelatin in comparison to the PLA control. Likewise, a clear radial O₂ gradient was observed emanating from the SONIC sample, whereas only a top-down gradient was observed in the control PLA simulation (Fig. 3h and Supplementary Movie 4). More importantly, SONIC incorporation was predicted to equilibrate the surrounding gelatin within roughly 2 h, whereas equilibration in the control system was significantly delayed (Fig. 3i and Supplementary Movie Video 4). Mechanistically, this is because diffusive resistance to O₂ transport in the SONIC scaffold is negligible compared to that within the hydrogel, and thus a high Pₐ gradient is established between the SONIC scaffold and the hydrogel at their interface, driving O₂ transport into the hydrogel. The EPR measurement procedure was repeated with two additional control scaffolds chosen to verify the vital role of the air channels in facilitating high oxygen permeability. One control was a commercial porous sponge comprised of hydrophilic melamine, which was selected as a simple example to confirm that a liquid-filled porous material would not provide benefit to O₂ transport. The second control was a PVDF-HFP scaffold, modified by the following two-step process to render the internal microporous channels hydrophilic: (1) ethanol was added to the tris buffer during incubation with dopamine to allow buffer solution penetration into the micropores of the scaffold, therefore enabling the application of the hydrophilic polydopamine coating within the microchannels; (2) the scaffold was treated with radiofrequency plasma to further ensure its hydrophilicity. O₂ transport tests showed that these two scaffolds were significantly slower than SONIC at equilibrating the system to exposed O₂ levels (Supplementary Fig. 6a). Spatial O₂ distributions of the sample containing the hydrophilic porous PVDF-HFP showed a top-to-bottom O₂ gradient throughout the system until a steady-state was achieved (Supplementary Fig. 6b), rather than the radial gradient emanating from the SONIC insert (Fig. 3g), the latter of which enabled rapid and deep O₂ penetration to the bottom. The results from these two additional controls indicate the necessity of the non-wettability of the internal microchannels for enabling rapid O₂ transport in the SONIC system.

The SONIC device improves cell survival under hypoxic conditions. In vitro tests and a complementary theoretical analysis were performed to evaluate the advantages of the SONIC scaffold for improving the viability of hydrogel encapsulated cells (Fig. 4). A computational model was developed to predict the oxygenation of INS-1 cells (2.5 million cells per mL alginate) in the control device and the SONIC device (Supplementary Fig. 7a). The boundary Pₐ was set as 40 mmHg (Fig. 4b) to mimic the Pₐ level in an intraperitoneal cavity (Supplementary Fig. 7b). As expected, the control device showed large hypoxic regions in the center due to the limited O₂ transport in such a thick bulk hydrogel (Fig. 4a), with a steep Pₐ gradient decreasing from 40 mmHg at the device exterior boundary to ~3 mmHg in the device center, corresponding with a drop of O₂ concentration (c O₂) from 0.05–0.004 mM (Fig. 4b). On the other hand, all regions in the simulated SONIC device showed sufficient oxygenation throughout the entire device (Fig. 4c). The Pₐ was maintained high over 35 mmHg in the whole SONIC device with only a minor difference between central and surface regions of the cell/hydrogel phase. Correspondingly, the cell/hydrogel phase showed high cO₂ between 0.044–0.05 mM, while the SONIC scaffold showed a substantially higher equilibrium cO₂ (~1.85 mM) due to the preferential partitioning of O₂ into the gas-containing SONIC scaffold (Fig. 4d). This suggests that the penetrating redistribution of external O₂ by the SONIC scaffold should have a significant positive impact on cell survival, especially for deeply encapsulated cells.

Results from the in vitro test were consistent with model predictions. Cell-containing devices were incubated in a hypoxic incubator with 5% CO₂ and 5% O₂ (i.e., 40 mmHg). After culturing for 48 h, cell viability was evaluated using a live/dead staining kit. Cells in the central region of the control device were dead likely due to hypoxia, with surviving cells limited to a thin region near the surface (Fig. 4e). In contrast, robust cell viability was detected throughout the SONIC device, even in the device center (Fig. 4f). These findings validated model accuracy and more importantly provided proof-of-concept for incorporating
the SONIC scaffold into a device to overcome O₂ diffusion resistance to encapsulated cells.

The computational model was adapted to simulate O₂ profiles in SONIC devices, empty control devices, and control scaffold devices containing islets (Supplementary Fig. 8–10). In this model, 500 IEQ of rat islets were considered as discrete spheres, with diameters (30–300 µm) selected from a distribution—simulating the natural size heterogeneity of islets—and dispersed randomly within the alginate domain (Supplementary Fig. 8a, b). Cross-sectional surface plots show deficient pO₂ levels in the center of the control device (Fig. 4g), whereas pO₂ levels throughout the SONIC scaffold were maintained uniform and high at ~35 mmHg near that of the external source (i.e., 40 mmHg) to provide sufficient O₂ to the surrounding islets (Fig. 4h). Here, SONIC’s advantageous O₂ delivery mechanism is illustrated: external O₂ crosses only a thin barrier of the slow-diffusivity alginate before it reaches the SONIC scaffold, where it permeates rapidly throughout the structure, achieving a scaffold pO₂ level near that of the surrounding environment (Fig. 1d). This rapid equilibration is achieved because of the high O₂ permeability of the SONIC system, which is enabled by the bicontinuous air channels rather than the PVDF-HFP material itself (Supplementary Fig. 8d, e and Supplementary Table 1).

Quantification of the oxygenation of the islet population in devices showed that the average islet pO₂ in the SONIC device was 1.8-fold higher than that in the control device (Fig. 4i), reducing the fraction of necrotic tissue 5-fold compared to islets in the control device (Fig. 4j) and Supplementary Fig. 9g, i). Additional analysis indicated that SONIC device encapsulated islets exhibited pO₂ levels descending roughly linearly with their increasing diameter (Supplementary Fig. 9c), with only a few of the largest islets displaying small regions falling below pO₂ levels associated with the impaired function (<4 mmHg) or necrosis (<0.08 mmHg) in contrast, many islets, including some small ones, were anoxic in control devices (Supplementary Fig. 9d, e). These results indicate that significantly improving the rate of O₂
transfer throughout the system bestows substantial benefits to islet oxygenation.

The simulation was repeated, but with the adaptation that each islet was implemented as 150 µm in diameter (the standard size for the definition of an islet equivalent) to isolate the effect of islet distance from the surface (Supplementary Fig. 10). In the control device, it was observed that islets near the device surface were well-oxygenated, while those in the center were anoxic (Fig. 4k). On the other hand, islets at all positions in the SONIC device were reasonably well-oxygenated (Fig. 4l). This shows that islet oxygenation is highly dependent on encapsulation depth in exclusively hydrogel-based systems (e.g., the control device), and that SONIC scaffold incorporation effectively decouples islet oxygenation from proximity to the external O₂ source (i.e., the tissue surrounding the device in the host site).

The in vitro study and theoretical analysis performed here to confirm the benefit of the SONIC scaffold to improve O₂ delivery to encapsulated cells and elucidates its mechanism of action. As previously mentioned, it is often suggested that islets must be within a few hundred micrometers to the device-host boundary to avoid debilitating O₂ diffusion resistances\(^{19,20}\). We predict here that the pO₂ levels in the SONIC scaffold are near those available in the transplantation site (i.e., intraperitoneal cavity). The SONIC device was designed such that any islet is within 300 µm from the SONIC scaffold or the device-host boundary. It follows that every encapsulated islet is thus within 300 µm to a
high pO2 source, regardless of its distance to the host site tissue. This not only permits the construction of thick devices, but also should allow the incorporation of significantly higher cell densities without significant impact on cell oxygenation (Supplementary Fig. 7d, e and Supplementary Fig. 11), especially with human islets which feature lower O2 consumption rates\(^8\) (Supplementary Fig. 12), and mitigate negative outcomes should the external pO2 environment be lower (Supplementary Fig. 13). The computational analysis provided here, and corresponding in vitro tests, suggest that the SONIC device significantly improves cell survival in hydrogel-based devices.

**The SONIC device enables long-term diabetes correction in mice.** Following in vitro testing and theoretical analysis, in vivo studies were pursued to test the therapeutic capability of the SONIC device (Fig. 5). Rat islets (500 IEQ per transplant) were incorporated in the SONIC devices (Fig. 5a, b; ~20 mm in length and ~4 mm in diameter) or controls (bulk hydrogels of the same dimensions but without the SONIC scaffold) (Supplementary Fig. 14) and transplanted into the intraperitoneal cavity of diabetic C57BL/6 mice. While both groups achieved normoglycemia within a few days after transplantation, the BG levels in the control group gradually rose and reverted to hyperglycemia after couple of weeks (Fig. 5c). H&E staining of the retrieved control sample showed the islets were necrosed with severe karyorrhexis or complete loss of nuclei (Supplementary Fig. 15). We speculate that the progressive failure of control devices was mainly due to initial hypoxia and necrosis of islets in the center of the device, resulting in the release of danger-associated molecular patterns (DAMPs), and subsequent damage to peripheral islets from DAMP-induced immune activation\(^5\). In contrast, the BG readings in the SONIC group were under control for almost 6 months (Fig. 5c). BG returns to hyperglycemia after device retrieval confirmed that the function of the device was responsible for diabetes correction.

Intraperitoneal glucose tolerance tests (IPGTT) were conducted on day 60 (Fig. 5d) and day 180 (Fig. 5e) before retrievals to evaluate the kinetics of insulin release from the devices. The SONIC group showed a well-preserved glycemic profile similar to that of healthy mice: the BG returned to normoglycemia within 2 h, indicating reasonable glucose clearance by the SONIC devices. By contrast, a poor glycemic profile was observed in the control group, as the BG remained at a high level similar to that of the diabetic control mice. A static glucose-stimulated insulin secretion (GSIS) test of the retrieved SONIC devices also showed glucose responsiveness of the encapsulated islets (Fig. 5f).

In addition, the live/dead staining revealed robust islet viability (Fig. 5g). H&E staining and insulin/glucagon immunostaining further confirmed the viability and function of islets in the retrieved devices. Both the peripheral islets (Fig. 5h) and central islets (Fig. 5i) in the SONIC device showed intact morphology and positive insulin and glucagon expression.

A transverse section of a retrieved device at 6 months confirmed that both the islets close to the surface and deep within the structure were viable (Fig. 5j). This was consistent with a selected transverse surface plot from the computational model, which suggested that islets in both central and peripheral regions would be highly oxygenated (Fig. 5k).

The foreign-body reaction (FBR) is a ubiquitous phenomenon for almost all foreign implants, which induces fibrosis deposition on the implant surface, occasionally leading to a complete coverage by the formation of a collagenous fibrotic capsule\(^2\). Deposited fibrosis restricts the mass transfer at the implant/host interface and affects the viability of the encapsulated cells. Alginate is one of the most widely used materials for cell encapsulation due to its good biocompatibility. In addition, it has been reported that alginate capsules or fibers larger than 1.5 mm in diameter have a significantly mitigated fibrotic response\(^2\).\(^\text{34}5,55\). The diameter of this SONIC device is around 4 mm which is suggested to mitigate the fibrotic response. In acellular studies, minimal cellular overgrowth was observed on the retrieved device surface (Supplementary Fig. 16). For the islet-containing devices, most regions of the retrieved devices remained free of fibrosis (Fig. 5i, j and Supplementary Fig. 17a), though a mild cellular deposition was observed on partial regions (Fig. 5h, l and Supplementary Fig. 17b–d). Nonetheless, the islets near regions with deposited fibrosis remained healthy and no necrosis was observed (Fig. 5l and Supplementary Fig. 17d). Our computational model was adapted to simulate the effect of fibrosis coverage, implemented by setting a no-flux boundary condition on one half of the device surface, simulating an extreme case where fibrosis is assumed to eliminate O2 transport entirely (Supplementary Fig. 18a). While the simulated fibrotic side of the control device was hypoxic, with islets exhibiting high levels of necrosis, the simulated islets in the SONIC device were adequately oxygenated at both the fibrotic side and the unblocked side (Fig. 5m and Supplementary Fig. 18b). Near homogeneous pO2 levels within the scaffold illustrate that the SONIC scaffold “carries” O2 from high pO2 regions to low pO2 regions over millimetre-scale distances, effectively redistributing the available O2 throughout the device.

**The SONIC system enables diabetes correction in mice with thick device.** Successful in vivo results of the ~4 mm diameter
device motivated the exploration of yet a thicker device design (Fig. 6). Our computational model was adapted to simulate the effects of increasing device size prior to fabrication and in vivo testing. A cubic (6.6 × 6.6 × 6.6 mm) control device and SONIC device were considered, each including 500 IEQ of size-distributed rat islets (Fig. 6a, b and Supplementary Fig. 19a). Remarkably, in silico islets in the 6.6 mm-thick SONIC device were similarly oxygenated (with similarly negligible necrosis levels) as the former 4.2 mm device due to the rapid O2 redistribution by the gas-phase transport through the SONIC scaffold (Fig. 6c, d). The simulation, repeated but with all islets being 150 μm in diameter (Supplementary Fig. 19b), showed that the pO2 of control islets decreased with distance from the external boundary (Fig. 6e), whereas even the innermost islets in the SONIC device exhibited high pO2 levels (Fig. 6f). This indicates that the advantage of the SONIC device (decoupling islet oxygenation from distance to the host site) was maintained even at this thicker scale.
We then fabricated a 4-layer scaffold using a new printed mold to test the model predictions (Supplementary Fig. 2A). A 6.6 mm-thick SONIC device was prepared using the same procedure as described earlier (Fig. 2A). Rat islets (500 IEQ per transplant) were incorporated into devices and transplanted into the infraperitoneal cavity of diabetic C57BL/6 mice (n = 10), with 5 retrieved at day 60 and the remaining retrieved at day 120 (Fig. 6g, h). Strikingly, all mice achieved normoglycemia within a few days after transplantation, with 4 out of the 5 mice in the long-term study maintaining normoglycemia for 4 months (Fig. 6i). Additionally, the BG returned to hyperglycemia after device retrieval, confirming the role of the device in diabetes correction. An IPGTT performed on day 58 showed that the BG level in device-treated mice was significantly lower than that in healthy control mice (Fig. 6j). BG monitoring was extended for 6 months in device-treated mice showing well-oxygenated islets in both peripheral regions and central regions of the device (Fig. 5). H&E staining of the transverse section of a retrieved device on day 181 showing healthy islets in both peripheral regions and central sections of the device. The asterisk indicates the host side of the device-host interface. One representative of 2 replicates is shown in Supplementary Fig. 22.

Discussion

Herein, we report the design and testing of an insect-inspired scaffold (SONIC) which featured internal continuous air channels for rapid O₂ delivery to hydrogel encapsulated cells. Incorporation of this scaffold into bulk hydrogels containing islets (the SONIC device) supported high islet viability and robust function even in devices with a thickness of 6.6 mm. The SONIC scaffold was comprised of a hydrophobic fluoropolymer PVDF-HFP, and the internal continuous air channels were created by a phase separation process. A hydrophilic polydopamine coating was applied to the scaffold surface to provide a compatible interface between the hydrophobic SONIC scaffold and hydrophilic hydrogel, while maintaining internal hydrophobicity to avoid water penetration into the air channels which were verified as essential for enabling the high O₂ permeability of the scaffold. Finally, the alginate hydrogel phase was interlocked with the skeleton of the SONIC scaffold to prevent hydrogel detachment during retrieval of the transplanted device.

O₂ supply is a primary limiting factor for cell encapsulation, especially for cells with high O₂ consumption rates such as islets. To overcome this limitation, much attention has been deservedly focused on exogenous O₂ supply, such as O₂-generating13–18 and O₂-filling devices13,14. However, O₂ transport within the hydrogel-encapsulated cell component is driven by gradients of O₂ tension and remains slow due to the poor O₂ permeability in aqueous media. Fewer efforts have focused on improving O₂ transport within the encapsulation domain itself. Some reports have explored the effect of perfluorocarbon (PFC) emulsion incorporation in hydrogels due to the high O₂ solubility56,57, and slightly higher diffusivity58 in PFC compared to hydrogels. However, these systems generally only yield modest benefits because of the limited improvement in O₂ permeability in composite systems where the phase with the higher permeability (in this case, PFC) is dispersed59. The bicontinuous gas-phase, endowed with extremely high permeability, incorporated into the hydrogel by the SONIC scaffold facilitates the rapid permeation of O₂ throughout the device, thereby enormously improving the effective O₂ permeability of the system. Regardless of design, the current SONIC system is reliant on the O₂ available in the transplantation site (Supplementary Fig. 13); however, we also posit that the SONIC scaffold could be used to further enhance O₂ supply to hydrogel encapsulated cells even in devices that provide exogenous supply.

The mechanism of improved O₂ distribution via SONIC can be visualized accordingly: the continuous hydrogel phase of the device can be considered as comprising small cubic regions of ~600 × 600 × 600 μm (Supplementary Fig. 22). Each of these hypothetical "microcubes" is adjacent to the SONIC scaffold which contains uniform pO₂ levels comparable to those of the transplantation site. Thus, it is as if each hypothetical microcube itself was implanted individually in the infraperitoneal space. Furthermore, the interconnection of the hypothetical microcubes ensured that other water-phase nutrients (e.g., glucose), found in...
the host site in significantly higher concentrations than O₂, permeate throughout the hydrogel phase.

A successful islet delivery implant must not only maintain cell survival but also ensure timely release of insulin to prevent postprandial hyperglycemia and overcorrection into hypoglycemia. The delay in IPGTT observed in mice treated with the thick SONIC device (Fig. 6j) indicate that, for islet delivery, the possibility of simply extending the device structure in all three dimensions may be limited. However, cellular treatments for hemophilia and liver diseases also utilize high cell payloads without the requirement of phasic therapeutic release. Therefore, the thick SONIC system may find broader utility in these applications.

We also emphasize that 3D printing enables the SONIC scaffold to be fabricated in scaled-up dimensions (e.g., in multiple layers or extended in length to tens of centimeters) in a wide range of designs (e.g., toroidal, spiral) (Supplementary Fig. 23 and Supplementary Fig. 24). An advantageous configuration of an islet delivery device is a planar hydrogel disk (1.2 mm thickness) incorporating an internal SONIC scaffold configured in an Archimedean spiral with a 500 μm gap between turns (Fig. 7 and Supplementary Fig. 25a). The small thickness of 1.2 mm obviates
limitations associated with delays in insulin release, and the spiral structure ensures that each islet is within 250 μm of a high pO2 source in the SONIC scaffold (Fig. 7a). We assessed the capacity of this construct to accommodate variable densities (4–10%) of human islets in comparison to a control construct without the SONIC scaffold using the computational model (Fig. 7b). Model predictions indicate significantly higher oxygenation of islets in the SONIC spiral device relative to the control construct, and negligible necrosis levels up to 8% (v/v) human islet loading density (Fig. 7c–f). If extended radially, this construct could support a curative islet dose of 500 k IEQ within a disk approximately 11 cm in diameter.

In summary, this work provides a solution to the poor transport of O2 in traditionally employed bulk hydrogels of cell encapsulation systems. SONIC’s mimicry of the insect tracheal system yields a cell encapsulation device that is amenable to increased cell density, fibrotic blockage, and most notably, substantially increased device thickness without sacrificing cell oxygenation, in effect, decoupling cell survival from its distance to the external supply. Ultimately, these advantages imparted by the SONIC scaffold represent a promising platform for translatable encapsulation devices requiring high cell payloads.

Methods
Materials. Poly(vinylidene fluoride-co-hexafluoropropylene) (PVDF-HFP, Mw = 455 kDa), Tris hydrochloride (Tris-HCl), sodium hydroxide, dopamine hydrochloride, sodium chloride (NaCl), calcium chloride dihydrate (CaCl2·2H2O), barium chloride dihydrate (BaCl2·2H2O), calcium sulfate dihydrate (CaSO4·2H2O),...
Nile Red, gelatin and D-glucose were purchased from Sigma-Aldrich. Poly(lactic acid) (PLA) filament was purchased from PRUSA. Ultrapure sodium alginate ( Pronova Biopolymer G2018) purchased from Novo Nordisk. Water was deionized to 18.2 MΩ cm with a Synergy UV purification system (Millipore Sigma).

Animals. Male C57BL/6j mice (2 months old) were purchased from The Jackson Laboratory. The mice were maintained at a temperature of 70–72 °F with 30–70% humidity under a 14 h light/10 h dark cycle. Male Sprague-Dawley rats (weight of ~300 g) were purchased from Charles River Laboratories. All animal procedures were approved by the Cornell Institutional Animal Care and Use Committee and complied with relevant ethical regulations.

Characterizations. High-resolution X-ray computer tomography (Nano-CT) scanning was conducted on a 3D X-ray microscope (ZEISS Xradia 520 Versa). Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) element mapping were performed using a field emission scanning electron microanalyzer (LEO 1530). Contact angle images were taken using a contact angle goniometer (Rame-Hart 500). Optical and fluorescent microscopy images were taken using a digital microscope (EVOS FL). H&E staining images were taken using an Aperio Scanscope (CS2). Stereo microscope images were taken by a stereomicroscope (Olympus SZ61). Immunofluorescence images were taken using a confocal microscope (ZEISS LSM 710). OriginPro 8.5.1 software and GraphPad Prism 8 software were used for data plotting.

Fabrication of the SONIC scaffold. PVDF-HFP was dissolved in acetone at a concentration of 15 wt% under heat in a sealed glass vial. After cooling to room temperature, the PVDF-HFP solution was filled into a 3D printed PLA mold (OriginPro 8.5.1 software and GraphPad Prism 8 software were used for data plotting).

Nanocoating (zymoPlex) fabrication. The extracellular matrix proteins (ECM proteins) were isolated from the SONIC scaffold. First, the scaffold was fixed with 10% formalin, embedded in paraffin. The SONIC scaffold was retrieved from the mold and immersed in the buffer (95 mM CaCl$_2$+ 5 mM BaCl$_2$), leaving the device in a buffer around 4 min for further cross-linking. Constructs that contained INS-1 cells or insulin alginate solution to load the mold and immersed in the buffer (95 mM CaCl$_2$+ 5 mM BaCl$_2$) for cross-linking by the Ca$^{2+}$ and Ba$^{2+}$ ions which diffused through the dialysis membrane. Next, the tubing mold was unrolled to leave the alginate in the buffer for around 4 min for further cross-linking.

In vitro cell viability study. INS-1 cells were purchased from Sigma-Aldrich and cultured with RPMI 1640 medium (Gibco) supplemented with 10% FBS (Gibco), 10 mM HEPES (Gibco), 2 mM glutamine (Gibco), 1 mM sodium pyruvate (Gibco), 50 µM β-mercaptoethanol (Gibco), and 1% penicillin/streptomycin (Gibco). Trypsin-dissociated INS-1 cells were suspended in alginate solution at a density of 2.5 million cells/mL and incorporated into SONIC devices or control devices, and each was incubated in the above-mentioned medium in a hypoxic incubator with 5% O$_2$, 5% CO$_2$ at 37 °C. After 48 h, the cells in devices were stained with a LIVE/DEAD viability/cytotoxicity Kit (Invitrogen).

In vivo cell viability study. Male C57BL/6 mice (6–8 weeks old) were purchased from Charles River Laboratories. The mice were maintained at a temperature of 70%–72% humidity under a 14 h light/10 h dark cycle. Male Sprague-Dawley rats were purchased from Charles River Laboratories. All animal procedures were approved by the Cornell Institutional Animal Care and Use Committee and complied with relevant ethical regulations.

Implantation and retrieval in mice. C57BL/6j mice were anesthetized using 3% isoflurane in O$_2$ throughout the whole surgery. Briefly, the pancreas was dissected with 10% 0.15% Liberase (Roche) in M199 media (Gibco) through the bile duct. The pancreas was digested at 37 °C circulating water bath for ~28 min (digestion time varied slightly for different batches of Liberase). The digestion was stopped by adding cold M199 media with 10% FBS (Gibco). After vigorously shaking, the digested pancreases were washed twice with media (M199 + 10% FBS), filtered through a 450 µm sieve, and then suspended in a Histopaque 1077 (Sigma)/M199 media gradient and centrifuged at 1700 RCF at 10 min at 4 °C. The gradient centrifugation step was repeated for higher purity. Finally, the islets were collected from the gradient and further isolated by a series of gravity sedimentations, in which each top supernatant was discarded after 4 min of settling. Islet equivalent (IEQ) number of purified islets was counted by reported IEQ conversion factors. Islets were then washed once with islet culture media (RPMI 1640 supplemented with 10% FBS, 10 mM HEPES, and 1% penicillin/streptomycin) and cultured in this medium overnight before further use.

Morphology and immunohistochemistry of islets in retrieved devices. The retrieved devices were fixed with 10% formalin, embedded in paraffin, and sectioned into 5 µm sections. Hematoxylin and eosin (H&E) staining was performed by Cornell's Histology Core Facility. For immunofluorescent insulin and glucagon filtered back-projection in isotropic 64 × 64 × 64 cube with 0.66 mm voxel linear size.

Fabrication of the SONIC device. The SONIC scaffold was immersed into a dopamine solution (2 mg/mL in 10 mM tris buffer, pH 8.5) overnight to create a hydrophilic polydopamine coating on the scaffold surface. Subsequently, CaSO$_4$ was deposited onto the scaffold surface by dipping it into a CaSO$_4$ saturated solution (0.24 wt% in water) and then drying it at 80 °C until CaSO$_4$ crystals on the scaffold surface. Next, the scaffold was inserted into a glass tubing mold with sodium alginate (2%) solution. Alginate cross-linking then occurred by the Ca$^{2+}$ ions diffused from the CaSO$_4$. Finally, the SONIC device was pushed out from the tubing mold into a cross-linking buffer (95 mM CaCl$_2$ + 5 mM BaCl$_2$), leaving the device in a buffer around 4 min for further cross-linking. Constructs that contained INS-1 cells or islets alginate solution to load the mold and immersed in the buffer (95 mM CaCl$_2$+ 5 mM BaCl$_2$) for cross-linking by the Ca$^{2+}$ and Ba$^{2+}$ ions which diffused through the dialysis membrane. Next, the tubing mold was unrolled to leave the alginate in the buffer for around 4 min for further cross-linking.
staining, paraffin-embedded sections were deparaffinized in xylene and sequentially rehydrated in 100% ethanol, 95% ethanol, 75% ethanol, and PBS. Slides were then boiled in citric acid buffer (10 mM citric acid, 0.05% Tween 20, pH 6.0) for 30 min for antigen retrieval. After blocking with 5% donkey serum, primary rabbit anti-rat insulin (Abcam, ab68320, 1:200) and mouse anti-rat glucagon (Abcam, ab10988, 1:200) antibodies were applied and incubated overnight at 4 °C. After washing with PBS, Alexa Fluor 594-conjugated goat anti-rabbit IgG (ThermoFisher, A11037, 1:2000) and Alexa Fluor 488-conjugated donkey anti-mouse IgG (ThermoFisher, A21202, 1:400) were applied and incubated for 60 min. Finally, slides were washed with PBS, applied with antifade/DAPI, and covered with glass coverslips.

BG monitoring & intraperitoneal glucose tolerance test (IPGTT). Mouse BG levels were measured by a commercial glucometer (Contour Next EZ, Bayer) with a drop of blood collected from the tail vein. For the IPGTT, mice were fasted for 16 h and administered an intraperitoneal injection of 20% glucose solution at a dosage of 2 g glucose/kg mouse. BG levels were measured at 0, 15, 30, 60, 90, and 120 min following the injection.

Ex vivo static glucose-stimulated insulin secretion (GSIS) assay. Krebs Ringer Bicarbonate (KRb) buffer was prepared according to the following formula: 98.5 mM NaCl, 4.9 mM KCl, 2.6 mM CaCl₂·2H₂O, 1.2 mM MgSO₄·7H₂O, 1.2 mM KH₂PO₄, 25.9 mM NaHCO₃, 0.1% BSA (all from Sigma-Aldrich), and 20 mM HEPES (Gibco). The retrieved devices were incubated in the KRb buffer for 2 h at 37 °C, 5% CO₂. Devices were transferred and incubated in KRb buffer supplemented with 3.3 mM glucose, then 16.7 mM glucose for 75 min each. The buffer was collected after each incubation step, and insulin concentration was measured using an ultrasensitive rat insulin ELISA kit (ALPCO).

Computational modeling. Five general finite element models were created to calculate theoretical O₂ profiles in SONIC-enabled constructs and corresponding controls. In all models, O₂ tension (pO₂) was related to the concentration of O₂ (cO₂) by the Bunsen solubility, or equilibrium concentration of O₂ in a material i at 37 °C, αSO₂:

\[ \text{cO}_2 = \alpha_{SO_2} \cdot \text{pO}_2 \]  

(1)

In other words, O₂ partitioning was governed by Henry’s law where, in Eq. 1, αSO₂ represents the inverse of Henry's constant. Each model is described below.

Model 1 (Fig. 3h, i and Supplementary Fig. 5) simulated time-dependent oxygenation of the in vitro acellular test. Two domains were considered: a rectangular prism representing the SONIC scaffold or PLA control (2 x 2 x 23 mm), and a surrounding cylinder (8.6 mm diameter, 17 mm length), representing gelatin, with the scaffold located in the center of the gelatin (Supplementary Fig. 5a, b). To emulate the experimental set up, boundary conditions at the top face of the gelatin and exposed faces of the scaffold were implemented at a constant pO₂ of 40 mmHg, while no flux conditions were implemented on all other faces due to the O₂ impermeability of the glass test tube (Supplementary Fig. 7b). Steady-state pO₂ following the injection.

\[ \alpha_{SO_2} \frac{\partial (\text{pO}_2)}{\partial t} = -\alpha_{SO_2} \cdot D_2 \cdot \left( \frac{\partial^2 (\text{pO}_2)}{\partial x^2} + \frac{\partial^2 (\text{pO}_2)}{\partial y^2} + \frac{\partial^2 (\text{pO}_2)}{\partial z^2} \right) \]  

(2)

here, D₂ represents diffusivity of O₂ in domain i (i.e., SONIC scaffold, PLA, or gelatin) at 37 °C. As it was confirmed that a bicontinuous porous structure was maintained throughout the SONIC scaffold (Fig. 1j, k, Supplementary Fig. 3, and Supplementary Movie 2), this domain was modeled as a gaseous air phase. Therefore, αSO₂SONIC = 3.9 x 10⁻⁶ mol/(m³ Pa) rather represents the equilibrium O₂ concentration of air and was calculated using the ideal gas law. Solubility and diffusivity values for gelatin varied widely in the literature, thus values for alginates hydrogel were used, their physical and chemical similarity. The remaining solubilities were thus implemented as αSO₂gellan = αSO₂alginate = 9.3 x 10⁻⁶ mol/(m³ Pa)⁵⁹, and αSO₂PLA = 4.5 x 10⁻⁶ mol/(m³ Pa)⁶⁰, each obtained from the literature. Likewise, D₂SONIC = 1.8 x 10⁻⁶ m²/s⁶⁴, D₂gellan = D₂alginate = 2.7 x 10⁻⁶ m²/s⁶⁵, D₂PLA = 1.6 x 10⁻⁶ m²/s⁶⁶, also obtained from the literature, were implemented for O₂ diffusivities in the respective materials. A time-dependent study simulated over 80 h was performed with a time step of 5 mins.

Model 2 simulated steady-state O₂ transport in cylindrical constructs (4.2 mm diameter, 20.4 mm length) containing alginate-encapsulated INS-1 cells with cell densities from 1.0–8.0 million cells/mL. As it was confirmed that a bicontinuous porous structure was maintained throughout the SONIC scaffold (Fig S17a), oxygenation of 500 IEQ rat islets in cylindrical constructs of variable lengths (6.4 mm vs. 20.4 mm) and therefore cell densities was modeled using Michaelis-Menten kinetics and a step-down function. In this model, RO₂iDO₂i was only defined in the islet domains according to the following:

\[ \text{RO}_2 \text{iDO}_2 \text{i} = \frac{\text{VINS}}{\text{Km} + \beta \cdot \text{pO}_2 \text{i}} = \frac{\text{VINS}}{\beta \cdot \text{pO}_2 \text{i}} \]  

where \( \text{Km} = 0.81 \text{mmHg} \), \( \beta = 112.6 \), and \( \alpha = 0.40 \). These values were obtained empirically and were found to be similar to distributions observed in other animal islet sources. In model 3, steady-state O₂ transport in all cylindrical constructs (4.2 mm in diameter, 20.4 mm or 6.4 mm in length) containing alginate-encapsulated rat islets (Fig. 4f–i, k, and Supplementary Fig. 8–11, Supplementary Fig. 13, and Supplementary Fig. 18; 500 IEQ rat islets per device) or human islets with cell densities from 2.74–8.04% w/v in devices (4.2 mm in diameter, 22.6 mm in length) was modeled using perfect spheres and seeded randomly in the alginate domain. As a default, islet diameters, d, were randomly selected from a size distribution. Rat islet diameters were selected from a lognormal distribution (Supplementary Fig. 8b), with the probability density function given by:

\[ f(d) = \frac{1}{d \sqrt{2 \pi}} \exp \left( \frac{-(\ln(d/\beta))^2}{2 \alpha^2} \right) \]  

(3)

where \( \alpha = 1.5 \) and \( \beta = 105 \). In specified cases (Fig. 4k, l and Supplementary Fig. 10), islets were instead all generated with \( d = 150 \mu m \).

Steady-state pO₂ profiles were obtained by solving the diffusion-reaction mass balance equation (Eq. 3). Solubility and diffusivity in the islets were given by αSO₂islets = 7.3 x 10⁻⁶ mol/(m³ Pa) and P₀₂islets = 2.0 x 10⁻⁶ m²/s respectively. In this model, RO₂iDO₂i was only defined in the islet domains according to the following:

\[ \text{RO}_2 \text{iDO}_2 \text{i} = \frac{\text{VINS}}{\text{Km}_i + \beta_i \cdot \text{pO}_2 \text{i}} = \frac{\text{VINS}}{\beta_i \cdot \text{pO}_2 \text{i}} \]  

(4)

where \( \text{VINS} = 0.0340 \text{mol/(m³ s)} \) represents the O₂ consumption rate in rat islets and \( \text{VINS} = 0.0134 \text{mol/(m³ s)} \) in human islets respectively. Model 3 was used to predict islet oxygenation and necrosis in cylindrical constructs implanted intraperitoneally in mice (Fig. 4g–i), whereby a constant pO₂ of 40 mmHg was implemented on all external boundaries (Supplementary Fig. 8c). The effect of variable external boundary pO₂ was explored in Supplementary Fig. 13. This model was also used to test the hypothetical impact of alternative scaffold compositions, including PLA, solid PVDF-HFP, or porous PVDF-HFP/alginate in constructs (Supplementary Fig. 8d, e). Solubility and diffusivity coefficients were used for the alternative scaffold materials are listed in Table S1. Model 3 was also used to predict the influence of partial-O₂-free islets in the same constructs (Fig. 5k, m and Supplementary Fig. 18), modeled by a no flux condition implemented on one half of the exterior of a cylindrical device (SONIC versus scaffold-free control, each 20.4 mm length) containing 500 IEQ rat islets to imitate a severe case of blockage by a fibrotic layer (Fig S17a). Oxygenation of 500 IEQ rat islets in cylindrical constructs on variable lengths (6.4 mm vs. 20.4 mm) and therefore cell densities was also evaluated with this model (Supplementary Fig. 11). Finally, Model 3 was used to evaluate human islet oxygenation and necrosis in cylindrical devices (SONIC versus scaffold-free) at variable densities (Supplementary Fig. 12).

Model 4 simulated steady-state O₂ transport in two thick cubics (6.6 x 6.6 x 6.6 mm) devices, each containing 500 IEQ rat islets (Fig. 6a–f, and Supplementary Fig. 19): a scaffold-free control device and a SONIC device. A constant pO₂ of 40 mmHg was applied to all external boundaries. All physics implementations of Model 4 were identical to those of Model 3, except for the dimensions, which are defined in Supplementary Fig. 19a. b.
flux condition was imposed on the lateral face, as the modeled geometry is intended to represent only the central region of a device which would be extruded radially. All other physics implementations were identical to those of Model 3, except for the dimensions, which are defined in Supplementary Fig. 25a. All models were solved in COMSOL Multiphysics or COMSOL LiveLink for MATLAB. In Models 3–5, all calculations were repeated for at least 3 iterations, whereby the islets were reseected and repositioned at random each time. For all calculations, a mesh was implemented using COMSOL’s “Free Tetrahedral” program with the following settings: maximum element size of 100 µm, minimum element size of 1 µm, curvature factor of 0.3, resolution of narrow domains of 3.3, and maximum growth rate of 1.25. It was ensured that all results were independent of the mesh.

Statistics. All results are expressed as raw data or as mean ± SD. Data from random BG measurements (Fig. 5c) were analyzed via a one-way analysis of variance (ANOVA) where device treatment (e.g., control device or SONIC device) was considered a discrete factor and time was considered a continuous covariate. Here, data from two-month SONIC device-treated mice (pink) were compared to data from control-treated mice (black) between days 4 and 61. Data from 6-month SONIC device-treated mice (red) were compared to data from control-treated mice (black) between days 4 and 181. Data from IPGTT studies (Fig. 5d, e and Fig. 6d) were analyzed via two-way analysis of variance (ANOVA) where both time and treatment (e.g., diabetic control mice, healthy control mice, control device-treated mice, and SONIC device-treated mice) were considered discrete factors, followed by a Sidak’s post hoc p-value adjustment for multiple comparisons. Data from the GSIS test (Fig. 5f) was analyzed via a paired two-tailed students t-test. Average population Po2 and necrotic fraction data from modeling studies (Fig. 4i, j) were analyzed via an unpaired two-sided students t-test. Data from modeling studies of the thick hydrogel (Fig. 6c, d), variable scaffold compositions (Supplementary Fig. 11d, e), variable density studies (Fig. 7c, d and Supplementary Fig. 12), and variable external Po2 (Supplementary Fig. 13) were analyzed via a two-way ANOVA followed by Sidak’s post hoc p-value adjustment for multiple comparisons. Statistical significance was concluded at p < 0.05.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
All data supporting the findings of this study are available within the article and the Supplementary Information files and from the corresponding author upon reasonable request. Source data are provided with this paper.

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Author contributions

L.-H.W. and M.M. conceived and designed the experiments. L.-H.W. and D.A. performed the experiments. L.-H.W. and A.U.E. performed the data analysis. A.U.E and A.K.D. performed the computational modeling. B.E. and M.K. contributed to the EPR O2 imaging. L.-H.W., A.U.E., and M.M. wrote the manuscript.

Competing interests

B.E. discloses financial interests in O2M Technologies. L.-H.W., A.U.E., and M.M. are inventors on a patent (no. US 63/174,739) based on this work filed by Cornell University on April 14, 2021. All other authors declare no competing interests.

Additional information

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