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Supporting Survival of Transplanted Stem-Cell-Derived Insulin-Producing Cells in an Encapsulation Device Augmented with Controlled Release of Amino Acids

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Abstract

Pancreatic islet transplantation is a promising treatment for type I diabetes, which is a chronic autoimmune disease in which the host immune cells attack insulin-producing beta cells. The impact of this therapy is limited due to tissue availability and dependence on immunosuppressive

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Supporting Information

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

Conflict of Interest

The authors declare the following competing financial interest(s): T.A.D. is a scientific founder of Encellin Inc., a cell therapy device company. Q.T. is a consultant and M.H. is on the scientific advisory board of Encellin. The University of California, San Francisco (UCSF) has filed a provisional patent application on this macroencapsulation technology for cell-based therapy.

drugs that prevent immune rejection of the transplanted cells. These issues can be solved by encapsulating stem cell-derived insulin-producing cells in an immunoprotective device. However, encapsulation exacerbates ischemia, and the lack of vasculature at the implantation site post-transplantation worsens graft survival. Here, an encapsulation device that supplements nutrients to the cells is developed to improve the survival of encapsulated stem cell-derived insulin-producing cells in the poorly vascularized subcutaneous space. An internal compartment in the device is fabricated to provide zero-order release of alanine and glutamine for several weeks. The amino acid reservoir sustains viability of insulin-producing cells in nutrient limiting conditions in vitro. Moreover, the reservoir also increases cell survival by 30% after transplanting the graft in the subcutaneous space.

Keywords

beta cell replacement therapy; cell encapsulation device; nanotechnology; transplantation; type 1 diabetes

Type 1 diabetes (T1D) is a tissue-specific autoimmune disease characterized by the infiltration of lymphocytes into the islets of Langerhans resulting in the loss of insulin producing beta cells, which leads to hyperglycemia. T1D can be cured by replenishing the beta cells in the patient through islet transplantation.[1–2] For the past several decades, the availability of beta cells has been limited because the primary source of beta cells is islets isolated from deceased donors. In the future, renewable sources of beta cells such as stem cell-derived beta cells or genetically engineered pig islets may solve the problem of limited donor organs.[1,3–5] However, the need for immune suppressive therapies to prevent the rejection of transplanted cells from immune-mediated rejection could hinder the wide application and long-term success of the beta cell replacement therapy.[6–8] Therefore, encapsulation devices have been devleoped to protect cells from the immune system and circumvent the need of immunosuppressive drugs. Macro- and micro-encapsulation devices provide immune protection to the encapsulated cells by physically preventing host immune cells from interacting with the grafted beta cells.[9–12] The physical barrier, however, limits the diffusion of gas and nutrients, especially before the device is completely vascularized (Figure S1, Supporting Information) [8,13] For this reason, cell survival within encapsulation devices is a significant obstacle that is limiting the success of employing encapsulation technologies for beta cell replacement therapy.

Previously, a nanoporous polycaprolactone (PCL) macroencapsulation device developed in our laboratory, has shown to be conducive to cell survival if implanted on the surface of the liver.[13,14] However, this transplantation site is invasive and difficult to monitor.[15] A much more preferred site for transplantation of experimental cell source such as stem-cell derived beta cells would be the subcutaneous space, which is a minimally invasive, accessible, and retrievable site. A drawback to this implantation site is that it is poorly vascularized and does not maintain viability and function of islets as well as the richly vascularized liver capsule. [16–18] We have shown previously that hypoxia and nutrient deprivation, consequences of ischemia, synergistically kill stem cell-derived insulin-producing cells. Moreover, supplementation of single amino acid, particularly alanine and glutamine, effectively

rescued beta cells from nutrient deprivation[19] Therefore, in this study, we present an improvement in the encapsulation device by fabricating a compartment that releases amino acids within the encapsulation device to sustain graft viability after transplant.

Using the fabrication technique described in previous literature, thin-film nanoporous and nonporous films were fabricated.[13] Nanoporous films, with pores ranging from 200 nm to 1 μm, were used for the encapsulation device. The pores in these outer membranes are large enough to allow transport of small molecules and peptides and yet small enough to prevent immune cells from penetrating and attacking the encapsulated cells.[13,14] These pores were generated by leaching PEG from PEG:PCL films, which is advantageous as it allows for tunable pore size and distribution.[20–24] To provide better control over the release rate, nonporous PCL films were used to create the small nutrient reservoir.[25] To build this reservoir, ≈10 mg of dry amino acid powder was encapsulated between two nonporous films, and the films were sealed using resistive heating (Figure 1a). The amino acid reservoir was then sandwiched between two nanoporous films, and the assembly was sealed, leaving a small opening available for a cell loading port (Figure 1b).

Once the encapsulation device with the nutrient-supplying internal compartment was assembled, cells were loaded in fresh medium, and the cell loading port was sealed to create the final, implantable device (Figure 1c). The resulting encapsulation device was reinforced by the addition of a thicker, nonporous backing layer which was heat-sealed to the rim of the device so that the device would maintain its shape after implantation. Overall, the encapsulation device is 1.8 cm in diameter while the amino acid device is 0.7 cm in diameter (Figure 1d). The cross section scanning electron microscopy (SEM) images of the nanoporous (Figure 1e) and nonporous films (Figure 1f) further confirm the porosity and successful fabrication of the desired films.

To support beta cell survival after transplant, steady release of amino acid is needed for at least 2 weeks since that is the time it takes for device vascularization to reach its plateau.[13] Diffusion rate of the amino acids from the reservoir was controlled by manipulating film thickness.[22,26] Films were fabricated with thicknesses of 10.7 ± 0.8 μm, 24.3 ± 3.7 μm, and 37.8 ± 1.7 μm, and the release of alanine and glutamine from devices was monitored in vitro for 18 days. ≈10 μm films released alanine at a rate of 203.1 ± 56.4 μg per day, whereas ≈25 μm and ≈37 μm films released alanine at 116.9 ± 32.6 μg per day and 54.6 ± 20.6 μg per day, respectively (Figure 2a). Similarly, glutamine was released at a rate of 162.7 ± 73.6 μg per day for the ≈10 μm films, 73.2 ± 34.3 μg per day for the ≈25 μm films, and 43.7 ± 14.4 μg per day for the ≈37 μm films (Figure 2b). The linear regression of the cummulative release across all devices showed R^2 values of ≈0.99, thus confirming the linear zero order release of amino acids from these devices (Table S1, Supporting Information). As expected, the release rates of the devices increased proportionally with membrane thickness regardless of the amino acid used (Figure S2, Supporting Information). Also, across all devices, the release profile shows zero order kinetics for over 2 weeks, which attests to the ability of the devices in providing consistent amount of sustained release (Figure S3, Supporting Information). ≈10 mg of each amino acid was encapsulated in the devices, and by 18 days, 36.1% of alanine and 29.2% of glutamine were released from the thinnest 10 μm films,

confirming that the device will provide a sustained released for at least 2 weeks (Figure S4, Supporting Information).

Although the average loading amount of alanine and glutamine was similar in all the reservoirs, the release rates of these amino acids were different. The higher release rate of alanine was expected since alanine is more lipophilic and has a lower molecular weight (89.09 g mol^{-1}) than glutamine (146.01 g mol^{-1}). This further shows that with the knowledge of critical parameters such as properties of the membrane and the encapsulated drug, one can roughly predict the release rate from the reservoirs and easily manipulate the reservoirs to achieve the desired rate of release.[25,26] Alternatively, other groups have looked at changing porosity to control the release rate; however it is not applicable to this study since amino acids are small molecules that diffuse rapidly through porous thin-film membranes.[22]

After demonstrating sustained release for more than 2 weeks, in vitro tests were performed to determine the effectiveness of the amino acid devices in increasing viability of stem cell-derived beta cells when placed under nutrient deprivation. To ensure the best survival conditions for the cells, reservoirs made with 10 μm films were used as they provide the highest rate of release. Cells were placed in wells containing either nutrient rich, regular media (RM) or nutrient lacking, deplete media (DM). Cells were also incubated in deplete media containing either 10 mM of free, dissolved amino acid or amino acid devices. After both, 24 and 48 h, the results show that in the presence of amino acid devices, there is a significant decrease in beta cell death when compared to cells cultured in nutrient-depleted media alone (Figure 2c,d). Moreover, at 24 h, this viability benefit is equivalent to using nutrient replete media and at 48 h, the viability benefit is significantly higher than using nutrient replete media. This not only indicates that alanine and glutamine are important in enhancing cell viability but also that the release rate from the 10 μm thick reservoirs is sufficient in providing a survival benefit. Cell viability was also tested at 2 weeks, and the results again demonstrated that there was increase in cell viability in the presence of amino acid reservoirs compared to both nutrient replete and deplete media (Figure 2e). Although the data are not statistically significant, it is still promising since it is not expected for cells to last for more than 3–4 days in nutrient-deprived conditions in culture. The lower percent cell death at the 2-week time point is due to the fact that 10× diluted media was used instead of the 100× dilution used for the short-term experiments. Also, since all the experiments were performed with cells from different batches, the batch-to-batch variability of stem cell-derived beta cell differentiations might have led to further fluctuations of cell death absolute numbers across all the in vitro assays.

To assess the ability of the amino acid releasing reservoirs in sustaining beta cell survival after transplant, luciferase-expressing stem cell-derived insulin-producing cells were encapsulated into various PCL devices and transplanted in the dorsal subcutaneous space of NOD.Cg-Prkdcscid Il2rgtm1Wjl/SxJ (NSG) mice (Figure 3a). Survival of the encapsulated beta cells after transplant was assessed in longitudinal studies by monitoring luciferase activity via bioluminescence imaging (Figure 3b). We measured the bioluminescence signal intensity associated with the graft starting immediately after transplant on day 0 and throughout a 21-day period. When stem cell-derived beta cells were encapsulated alone, the

graft rapidly lost its bioluminescent signal within the first few days, and ≈0% of cell survived at day 21. With the addition of the amino acid reservoir, graft survival significantly improved. When alanine reservoir was present, graft survival showed improvement up to 17.5%. Glutamine reservoirs showed similar graft protection with cell survival persisting at 19.8%. When both alanine and glutamine were added to the reservoir, graft survival at day 21 increased to 33.3% (Figure 3c). Moreover, after 1 month, the thin film devices and those containing amino acid reservoir were explanted along with the surrounding tissue, and H&E staining and immunostaining were performed (Figure 3d). The cross-sectioned H&E tissue staining shows no deposition of fibrotic tissue along the graft, showing the in vivo biocompatibility of the thin-film devices. The immunostaining shows that in the presence of amino acid reservoir, the GFP expressing insulin producing cells were encapsulated within the thin-film device. This further shows that encapsulation devices containing amino acid reservoirs help increase survival of encapsulated cells when tranplanted subcutaneously.

Together, these results show that the fabricated encapsulation device with a nutrient-releasing internal compartment substantially increases cell viability in vitro and in vivo. Engraftment of beta cells in the subcutaneous space has been a challenge due to the inherently low vascularization present in this area, which results in prolonged ischemia of the graft and high percent of cell death post-transplantation.[16–18] To prevent ischemia, encapsulated cells can be supplied with nutrients, alanine, and glutamine in particular, until the blood supply at the transplant site is restored.[19] The challenge is to design a device which will contain an internal compartment that can provide a steady and sustained supply of nutrients directly to the encapsulated cells while vascularization occurs. The conducted studies show that amino acid devices fabricated from 10 μm thick nonporous PCL membranes provide sustained release of both alanine and glutamine for more than 2 weeks, at constant rates of 203.1 μg per day and 162.7 μg per day, respectively. The amino acid reservoirs provided greater than 80% viability of cells, during, both, short term (24, 48 h) and long term (2 weeks) in vitro nutrient deprivation challenge. The in vivo results also showed that the amino acid devices increased survival of grafts to 17.5% and 19.8% when single amino acid reservoirs were added alone, and cell survival was up to 33.3% in the presence of a reservoir containing, both, alanine and glutamine. The lower rate of survival with amino acid supplementation observed in vivo when compared to the in vitro results is likely due to the additional hypoxic stress experienced by the cells in the in vivo condition that is not addressed. Previously, we have shown that optimal beta cell survival in vivo required prior adaptation of the beta cells to lower oxygen tension and amino acid provision.[19] Therefore, in the future, we can potentially increase graft survival in these devices by preconditiong cells to survive at low oxygen levels.

The device design approach utilized here allows for flexible design, tunable scaling, and manipulation of membrane properties. Characterization of these devices also demonstrated the predictability of the model through knowledge of drug properties, membrane thickness, porosity, and drug payload. To further enhance the device and increase the viability of encapsulated cells, multiple internal and/or external compartments can be fabricated to release various molecules, such as immunosuppressive drugs, hormones, molecules that promote stem cell differentiation, or a more refined cocktail of nutrients to sustain cell viability for a longer period. Other approaches include expediting the vascularization of the

devices by releasing angiogenic molecules, such as VEGF and/or releasing anti-inflammatory molecules such as IL1RA from an external compartment to protect the graft from immune response post-transplantation. [2,8] The concept developed herein is applicable to many cell encapsulation technologies, and further enhancement of these devices can be scaled for clinical applications to treat T1D.

Results are expressed as the mean ± standard error (SE) with a minimum of three replicates for each condition. Statistical analyses were performed using GraphPad Prism software. Two-way analysis of variance (ANOVA) and multiple comparisons with Holm–Sidak test were performed. Differences between conditions were considered to be statistically significant if $p < 0.05$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Mice use in this study were housed and handled according to ethical guidelines approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California, San Francisco, Committee on Laboratory Animal Resource Center. G.S.C. and G.F. contributed equally to this work. G.S.C., G.F., Q.T., and T.A.D. designed experiments. G.S.C. fabricated membranes and performed in vitro experiments in characterizing membranes. D.A.B. performed SEM analysis using the Scanning Electron Microscopy facility at San Francisco State University. C.J. and A.V.P. performed differentiation of stem cell-derived beta cell clusters. G.S.C. and G.F. performed in vitro cell viability experiments. G.F. performed in vivo experiments of cell encapsulation implants. G.S.C. and G.F. analyzed the data and wrote the manuscript. M.H., Q.T., and T.A.D. provided direction, supervised the project, and edited the manuscript. This work was supported by CIRM grant (DISC2-09559) and partially supported by JDRF.

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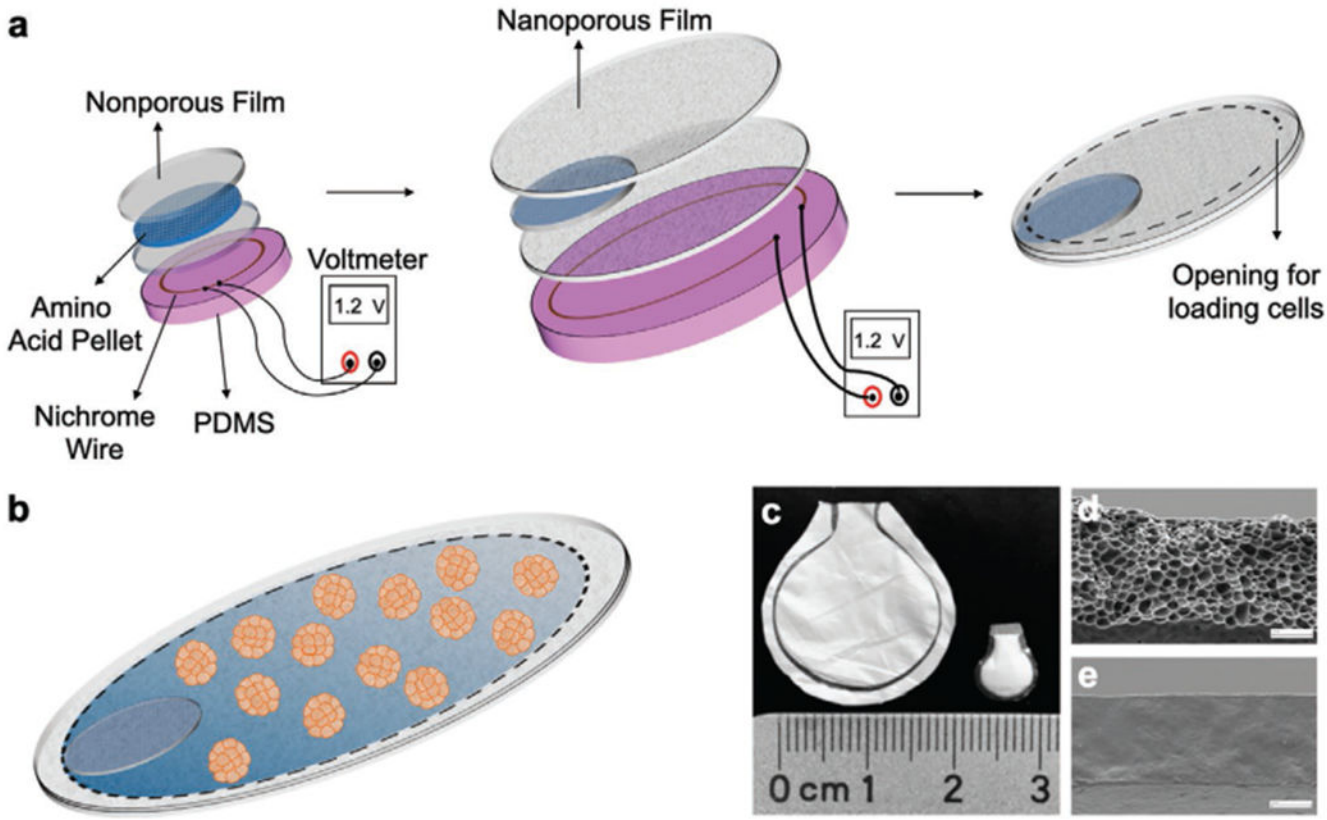

Figure 1.

Schematic showing the fabrication of encapsulation device with internal compartment. a) Amino acid devices were created by encapsulating a formulation of dry amino acid powder inside nonporous films and was sealed by the current flowing through the nichrome wire. The amino acid device was sealed within the interior of the encapsulation device by sandwiching the device along the edge of the encapsulation device membranes. The encapsulation device was sealed in a U shape (indicated by the dotted line) between two nanoporous films. Using a 200 μL pipet tip, cells and medium were added, after which the opening was heat-sealed. b) Cartoon illustration of sealed encapsulation device containing the internal amino acid reservoir. c) Image of assembled amino acid device (left; 0.7 cm) and encapsulation device (right; 1.8 cm). Cross section SEM of d) nanoporous and e) nonporous thin-films of ≈10 μm in thickness (scale bar = 10 μm).

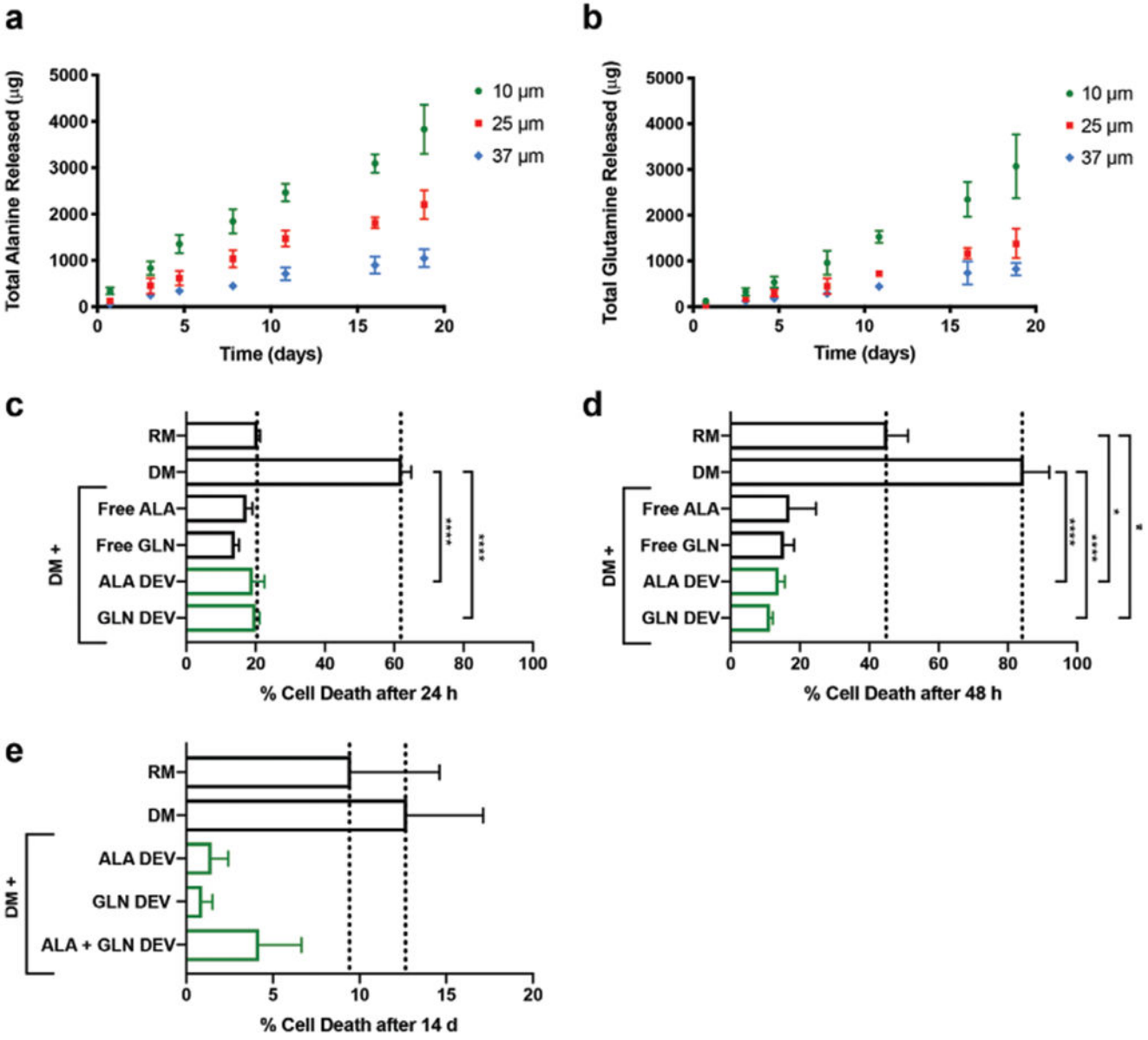

Figure 2.

In vitro evaluation of amino acid reservoirs in providing release and increase in cell viability. Sustained release of a) alanine (ALA) and b) glutamine (GLN) from thin film devices made with membranes of thicknesses varying from 10, 25, and 37 μm. Cumulative release of amino acids (μg) measured over the course of 18 days in PBS at 37 °C ($N = 4$ for each thickness; error bars represent ± SE relative to the mean). Cell survival benefit with ALA and GLN devices in depleted media (DM) compared to replete media (RM), DM (1:100 dilution of RM in PBS), and dissolved ALA and GLN in DM (free ALA, free GLN, respectively), over the course of c) 18 h, d) 48 h, and e) 2 weeks. Propidium iodide staining used to measure the decreased cell death shown in the presence of amino acid devices compared to DM ($N = 3$ per condition). Significance of differences of graft survival versus device control groups was determined using multiple unpaired *t*-test, corrected for multiple

comparison using Holm–Sidak method (error bars represent ± SE relative to the mean; *$p < 0.05$; ****$p < 0.0001$). Additionally, for the 2-week follow-up experiment, DM was changed to 1:10 dilution of RM in PBS in order to ensure long-term cell survival.

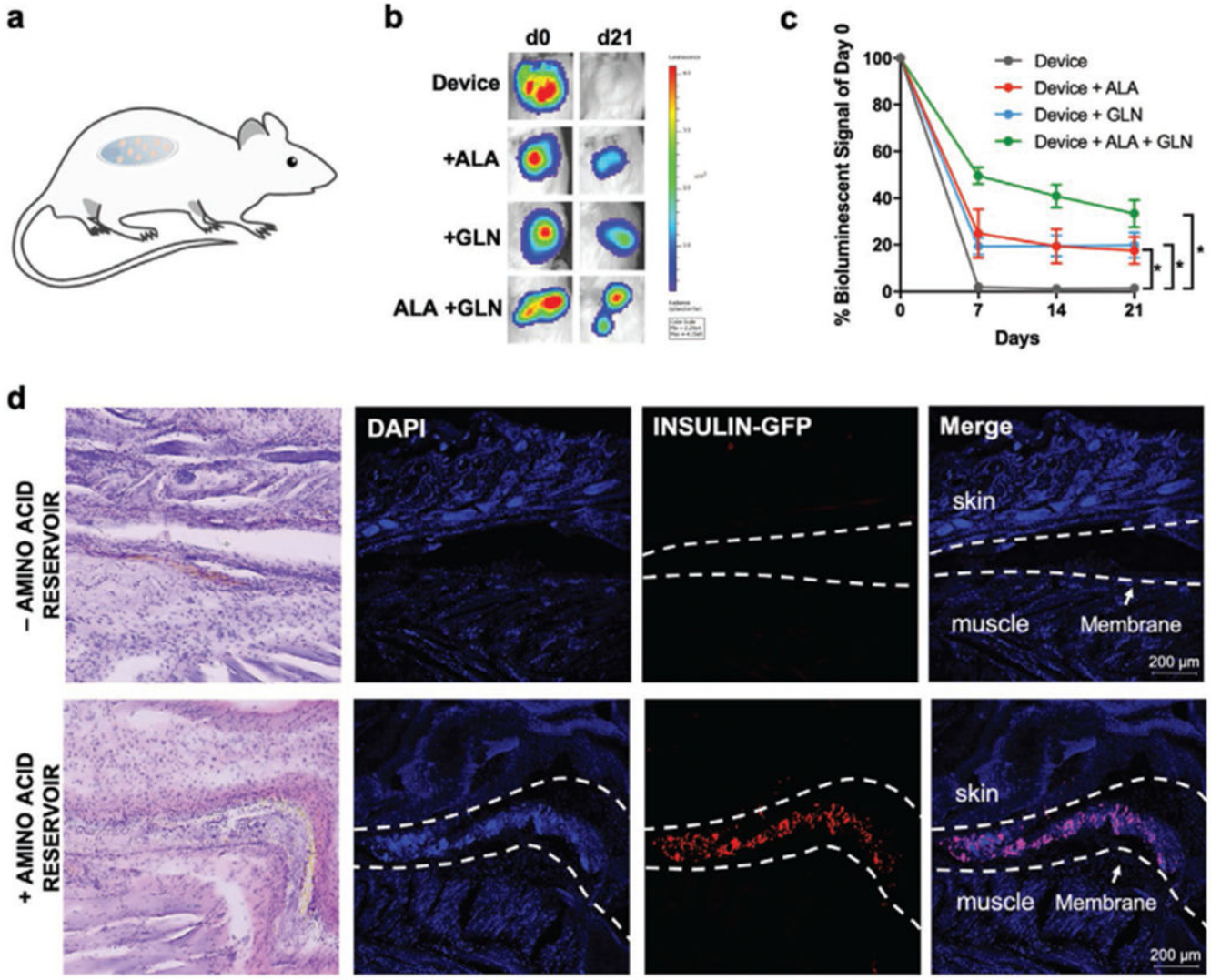

Figure 3.

In vivo viability of encapsulated cells in the presence of amino acid devices. a) PCL device transplanted in the subcutaneous space of NSG mice. b) Representative images of encapsulated SCIPC.LUC in PCL devices alone ($N = 7$), device + ALA reservoir ($N = 6$), device + GLN reservoir ($N = 6$), and device + ALA + GLN reservoir ($N = 6$). c) Quantification of bioluminescent signal of cells transplanted into encapsulation devices with or without amino acid reservoirs. Significance of differences of graft survival versus device control groups was determined using multiple unpaired *t*-test, corrected for multiple comparison using Holm–Sidak method (error bars represent ± SE relative to the mean; $*p < 0.05$). d) H&E staining and immunofluorescent staining of tissue sections of encapsulation devices with and without amino acid reservoir,obtained from NSG mice 21 days post-transplantation. Nuclei are visualized by DAPI staining and insulin-producing cells are genetically modified to express GFP. The dotted white lines delineate the perimeter of the thin-film encapsulation devices. Magnification 10×.