10c Improving Culture of Islets of Langerhans: Removing Oxygen Limitations

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Introduction: Recent success has made islet transplantation a promising treatment for type I diabetes mellitus. Several islet transplant centers culture islets following isolation from the donor pancreas. Insufficient oxygen transfer to islets in culture causes them to lose viability and function. In 3 mm of medium at 37˚C with 95% air/5% CO_2, surface coverage of <0.4% (21 IEQ/cm^2) prevents oxygen supply limitations but is impractical for clinical use. High-density islet culture is desirable because it reduces space and handling requirements during culture prior to transplantation, but it exacerbates oxygen limitations and causes a reduction in islet viability. We increased oxygen transfer to islets in culture either by culturing islets on an oxygen-permeable silicone rubber membrane or by culturing islets in an elevated pO_2. At the point of contact of a 150-μm diameter islet with the membrane surface, the pO_2 drop is less then 5 mmHg across a 25 μm membrane and 100 mmHg across a 500 μm membrane. At a surface density of 100 IEQ/cm^2 the pO_2 drop across 3 mm of medium is 105 mmHg, resulting in hypoxic regions in the islets, which increase with increasing surface density. Additionally, we solved the diffusion-reaction species conservation equation using finite element analysis to model oxygen transport for experimental culture conditions to determine theoretical oxygen profiles for culture at steady state. We hypothesized that increasing oxygen transfer by culturing on silicone rubber or in elevated oxygen will allow islets to maintain a higher viability at higher densities compared to a solid bottom dish. Methods: Human islets were cultured at densities varying from 21 IEQ/cm^2 to 5300 IEQ/cm^2 on either 500 μm silicone membranes or solid bottom dishes in a humidified incubator with 5% CO_2 at 37ºC with 19% or 56% oxygen. Culture time varied from 34-60 hr. Viability was assessed using oxygen consumption rate (OCR) measurements which were normalized with nuclei measurements. Theoretical oxygen profiles were determined using numerical calculations based on the species conservation equation solved by the finite element method. Results: The total viable tissue recovered after high density culture (as measured by total OCR) and the normalized OCR is higher when islets are cultured on silicone rubber versus oxygen-impermeable dishes. Silicone rubber also supports a higher OCR/area then an oxygen-impermeable dish, thereby allowing it to support a larger amount of viable tissue. Islets cultured at high density (>0.4% coverage) in 56% oxygen (428 mmHg) have a higher viability and result in a high viable tissue recovery following culture than islets at the same density in 19% oxygen (142 mmHg). Theoretical predictions for oxygen transport during culture predict trends similar to experimental results, but tend to over predict the amount of viable tissue recovered. Conclusions: Culture on a silicone membrane or in an elevated pO_2 increases oxygen transfer to the islet surface allowing islets to maintain a higher viability at high-density than they otherwise would on an oxygen-impermeable dish at normal pO_2.