

428m Controlled Expression of Insulin from Genetically Modified Tissue Engineered Skin Substitutes for Treatment of Diabetes

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Diabetes results from insufficient amount or complete lack of insulin in the bloodstream to mediate glucose uptake in the body. Although insulin can be administered by injection or other means the lack of control of insulin levels leads to long-term complications such as blindness or kidney problems. One approach to address this problem is to genetically modify non-beta cells to produce insulin. The skin is the largest and most accessible organ and as such it has high potential as an ectopic site for insulin delivery. Previously, we transduced human keratinocytes with a gene encoding for a mutated form of proinsulin (proinsulin sequence with two furin consensus sequences at the B-C and A-C junction), and showed that epidermal keratinocytes can process proinsulin and secrete mature insulin both in traditional cell culture and in the three-dimensional context of bioengineered skin substitutes. To further increase the level of insulin production, we introduced a point mutation in the insulin gene that has been found increase insulin production in pancreatic tumors. In addition, we transfected our modified cells with a gene encoding for furin, and showed that insulin production was enhanced by 2-fold. To further increase the furin cleavage efficiency, we modified the furin consensus sequence and examined the effect of insulin production from cells that were transduced with these constructs. Finally, we cloned the gene that produced the optimum level of insulin into a tetracycline regulatable vector. With this vector we were able to show transcriptional control of insulin gene expression by administration of doxycycline. Our results suggest that the epidermis can be modified as an ectopic site for regulated insulin delivery and that gene therapy of the skin may be used as an alternative strategy for the treatment of diabetes.