FUNCTION IN A RAT MODEL OF DILATED CARDIOMYOPATHY.

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Background: The plasma level of hepatocyte growth factor (HGF) is elevated not only in hepatic disease but also in myocardial injury.It is likely that HGF exerts protective effects against myocardial injury by its angiogenic and/or antifibrotic action. However, the effects of exogenous HGF on the cardiac function in failing heart have not been assessed sufficiently yet, because of its instability in the living system. We have prepared biodegradable gelatin hydrogels which achieve the controlled release of exogenous HGF over 2 weeks. This study is an investigation to evaluate whether the controlled release of exogenous HGF by the gelatin hydrogel of sheet type improved cardiac function in a rat model of dilated cardiomyopathy (DCM).
Methods and Results: Experimental autoimmune myocarditis was induced by immunization with porcine cardiac myosin in male Lewis rats. The rats that developed DCM with their fractional shortening less than 40 % were randomly divided into 2 groups 6 weeks later; Group I (10 rats): a gelatin hydrogel sheet (15 $\times$ 15 mm) incorporating 40 μg HGF was placed onto the epicardium of left ventricular (LV) free wall through left thoracotomy,and Group II (10 rats):a HGF-free hydrogel was applied in the same manner. Enzyme-linked immunosorbent assay (ELISA) 2 weeks after the surgery evaluated that the LV HGF levels were still high in Group I, compared with those in Group II. By echocardiographical assessment, the values of % fractional shortening were increased in Group I (56.2 $\pm$ 6.5%) compared with those just before the surgery (38 $\pm$ 5.2%), while there was no change in Group II (37.3 $\pm$ 5.5%). These findings indicated that LV systolic function was improved in Group I. The fibrotic area ratios assessed by Masson's Trichrome staining were lower in Group I than in Group II, both at the LV free wall (13.3 $\pm$ 1.2 vs 38.1 $\pm$ 3.6%, $p < 0.01$) and at the septum (10.3 $\pm$ 1.2 vs 24.2 $\pm$ 2.4%, $p < 0.01$). It is experimentally confirmed from sandwich ELISA that the levels of LV endothelin-1,the elevation of which closely correlates with the development of heart failure, were lower in Group I than in Group II, both at the LV free wall (9.6 $\pm$ 1.3 vs 19.8 $\pm$ 2.2pg/mL, $p < 0.01$) and at the septum (5.5 $\pm$ 0.7 vs 9.7 $\pm$ 1.2pg/mL, $p < 0.01$).
Conclusions: Epicardial controlled-release of HGF by gelatin hydrogel sheets improves LV systolic function in a rat DCM model. This functional improvement is attributable, at least in part, to the reduction of fibrosis and cardiac endothelin-1 synthesis.

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HIGHLY FUNCTIONAL TISSUE ENGINEERED HEART VALVES EVALUATED IN A LARGE ANIMAL MODEL

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BACKGROUND. The significant problems of long term durability and thrombogenicity associated with presently available heart valves necessitates a search for better alternatives using innovative approaches such as tissue engineering. Our previous attempts at creating heart valves in vitro have been hampered by (1) undue fragility of the developing tissues at the time of implantation, (2) the absence of sinuses of Valsalva in the wall of the construct and (3) a clinically unacceptable cell source. These problems have been addressed through a series of changes in the design of the heart valve scaffold and the identification of a novel source of cells. This study investigates the utility of these changes in a large animal model. METHODS. Mesenchymal cells were isolated from the bone marrow of sheep, characterized by immunocytochemistry and expanded in vitro. Three scaffold segments, each formed from biodegradable non-woven mesh and containing a single leaflet and sinus of Valsalva were joined along longitudinal seams to yield a 21mm valved conduit. Cells were delivered onto the scaffolds and cultured for a further period of 3–5 weeks. Valves were then implanted into the main pulmonary artery of adult sheep ($n = 6$, mean weight 64.5 $\pm$ 4.5 kg) on cardiopulmonary bypass, having first excised the native pulmonary valve leaflets. Handling characteristics of the tissue engineered structures were assessed qualitatively at implantation by the surgeon. Geometry of the valves and acute hemodynamic function were assessed in vivo by a cardiologist using epicardial echocardiography. RESULTS. The valve accepted sutures easily and did not tear during handling. The ratio of maximal sinus diameter to annulus diameter was 1.22 $\pm$ 0.03 Long axis views of the valve demonstrated three symmetrical valve leaflets and smooth continuity between the leaflets and their respective sinuses. Hemodynamic indices (mean $\pm$ SEM) were as follows. Mean systolic gradient 9.7 $\pm$ 1.3 mmHg, maximum systolic gradient 17.2 $\pm$ 2.0 mmHg, effective orifice area 2.1 $\pm$ 0.1 cm^2. Estimates of regurgitation ranged from trivial to mild. DISCUSSION. The tissue engineered valve handled very well surgically. The presence of sinuses, known to be important for long term functional durability of biological valves, was an important finding in this valve. Indices of acute hemodynamic function were equivalent or better than published values for commercially available heart valves of the same dimensions. CONCLUSION. This newly designed tissue engineered heart valve overcomes several problems experienced with previous grafts and provides a highly functional anatomical substitute for the pulmonary valve. Long term studies will be required to investigate the durability and event free survival of implanted tissue engineered heart valves.

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GEOMETRY OF HONEYCOMB COLLAGEN SCAFFOLD GIVES CHAMBER-TYPE MICROEMVIRONMENTAL UNITS FOR ECTOPIC OSTEOGENESIS WHEN IMPLANTED WITH PURIFIED BMP CAKTAIL INTO RAT SKIN

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To reconstruct local bone and periodontal tissues, we have proposed that five factors must be taken into consideration. They are (1) cells directly involved in the tissue formation, (2) natural and artificial matrices, (3) cytokines or signaling molecules and (4) vasculature and (5) biomechanical dynamics. In many clinical cases, it is not necessary to apply all five factors to the local lesion for reconstruction. Usually application of two or three factors is enough for successful reconstruction. To verify above proposition we have chosen BMP-induced ectopic osteogenesis, since this system essentially requires combination of cytokine and artificial matrices (carriers). Devising and examining more than ten different BMP-artificial matrices system, we concluded that geometry is crucially important for the bone related tissue engineering. Taxonomy of geometry proposed for all the known artificial matrices indicated that the concaved and tunnel-equipped geometries are feasible for bone formation (J Bone Joint Surgery, 83-A, S1-105–115, 2001). Recently we devised a unique biodegradable honeycomb collagen scaffold with numerous straight tunnels, diameters of which were controllable from 0.1 to 1.0 mm (Artificial Organs, 25:213–217, 2001). Moreover, this scaffold is mechanically tougher than ordinary sponge type collagen. Aim of the present study to analyze the mode of bone formation when the honeycomb collagen is used as BMP carrier. In this study we applied natural S300 BMP cocktail, which was purified from bovine bone by a 3-step chromatographic procedure, established in our laboratory, because this fraction contains all the BMPs of the bone-inducing activity, and thus may evaluate the maximal potency of a BMP carrier (J Biochem, 119: 475–481, 1996). The S300 BMP cocktail showed a highly reproducible bone-inducing activity when implanted with Insoluble bone matrix (IBM), that is the most effective BMP carriers ever reported. Honeycomb collagen (3 mg, disc form with diameter of 1 cm and thickness of 2 mm) was combined with 0.5 mg of S300 BMP cocktails and implanted subcutaneously into rat. Two weeks after implantation, histological observation clearly showed that cartilage and bone formation occurred adjacently with the individual honeycomb pores, which gave a quite different pattern of osteogenesis from that observed in the BMP/IBM system. But some pores were still rich in fibrous connective tissue, while the other pores were already filled with bone. Since each pore was separated by membranous wall of collagen, honeycomb collagen provided the chamber-like microenvironments for the sequence of events in endochondral ossification. Thus we can continuously analyze a certain stage in the process of osteogenesis in details. Calcium contents of the BMP/honeycomb collagen implants indicated an equivalent efficacy to the BMP/IBM system. In conclusion honeycomb collagen is characterized by compartmental ossification when used as a BMP carrier and useful for local bone and periodontal tissue engineering.

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CHARACTERIZATION OF POLY(HYDROXYBUTYRATE-CO-HYDROXYVALERATE)/TOOTHAPATITE COMPOSITE SCAFFOLD FOR BONE TISSUE ENGINEERING

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Tissue engineering is an interdisciplinary field that applies principles of engineering and the life science toward the development of biological substitutes that restore, maintain, and improve the function of damaged tissues and organs. In the field of tissue engineering using polymer scaffolds, development of suitable material for clinical application is a crucial factor. Although many trials have been undergone, there is no ideal materials for bone tissue engineering. The purpose of this study is to evaluate the physical properties of poly(hydroxybutyrate-co-hydroxyvalerate, PHBV) as a polymer matrix and calcined and pulverized human tooth (toothapatite, TA) as a novel, porous composite scaffold for bone tissue engineering. TA was almost composed of hydroxyapatite and whitelockite and the composite scaffold showed the interconnective open pore at both interior and exterior surface with 200–250 μm diameter. Mean porosity was 83.63 $\pm$ 1.5 %. The porosity was decreased in accordance with increase of amount of TA in the scaffold. The effects of TA in the scaffold were as follow; increase wettability of the scaffold, decrease the crystallinity, glass transition temperature, and melting temperature of the polymer, increase Young's modulus of the composite. In vitro biocompatibility of the scaffold was good in all specimens. These results suggest that the PHBV-TA composite scaffold, especially the scaffold containing 30 wt% of TA may be a good candidate for bone tissue engineering of non-load bearing area.

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RGDS-IMMOBILIZED TEMPERATURE-RESPONSIVE SURFACE FOR NON-SERUM CULTIVATION AND NON-INVASIVE CELL RECOVERY

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Appropriate surface chemistry methodologies for design of biomaterials, for applications in tissue engineering, have arisen in recent years. In developing tissue regeneration *in vitro*, one strategy is to recover the cultured cells to be maintaining their original functions as in body. We had already succeed in developing a novel cell recovery system using the intelligent culture dishes, on which temperature-responsive polymer is grafted. The grafted polymer, poly(*N*-isopropylacrylamide) (PIPAAm), has its original unique property that the wettabilities on the surfaces can be changed by temperature control. Therefore, it is able for cells cultured on the surfaces to lift up spontaneously by lowering temperature below the lower critical solution temperature (LCST) of PIPAAm (32 C) without enzymatic treatment, because the PIPAAm hydrates suddenly across the LCST and the surface demonstrates more hydrophilic to resist the non-specific protein adsorption and cell attachment In this study, we have immobilized the integrin-ligand peptide, Arg-Gly-Asp-Ser (RGDS), on temperature-responsive polymer-grafted surfaces to promote cell attachment and spreading under non-serum conditions. PIPAAm was functionalized by copolymerization with reactive co-monomer which possesses carboxyl group and these copolymers were grafted onto the cell culture dishes. RGDS peptides were immobilized on functionalized PIPAAm-grafted surfaces via carboxyl groups and these surfaces stimulated and promoted human umbilical vein endothelial cells (HUVECs) attachment and spreading, which depended on immobilized RGDS content. And even if cells were cultured without serum, these surfaces demonstrated good cell