numbers of CMs for therapeutic cell transplantation. This study investigated the effect of co-culturing with native CMs on ascorbic acid-induced cardiomyogenic differentiation in embryonic stem cells, to develop a novel protocol for generating functional CMs from ESCs.

Methods Native CMs were isolated from the hearts of 1-day-old Sprague-Dawley rats. Mouse ESCs were cultured in hanging drops to form embryoid bodies (EBs) and treated with or without 0.1 mM of ascorbic acid (Sigma) for cardiomyogenic differentiation. They were divided into four groups: ascorbic acid & co-culture group, co-culture group, ascorbic acid group, and control group. In the co-culture system, EBs were co-cultured with native CMs by the hanging cell culture inserts (PET 1 μm) (Millicell; Millipore, Bedford, MA, USA). The native CMs were purposely placed on culture plate inserts to prevent direct contact with subnatant EBs. Both the ESCs and native CMs grew in the same medium but they were easy to separate. The structural and functional properties of ESC-derived CMs (ESCM) were evaluated by microscopic observation, immunocytochemistry, RT-PCR, and transmission electron microscopy.

Results The average percentages of EBs exhibited rhythmic contractions in co-culture and ascorbic acid group, co-culture group, ascorbic acid group, and control group were 86.6±9.52%, 65.60±10.77%, 29.6±6.03%, and 17.76±5.99%, respectively. The percentage of beating EBs in co-culture & ascorbic acid group was much higher and the homogeneity of EBs were significantly improved over that seen in other groups ($p<0.01$), simultaneously, the automaticity of beating also maintained for more time. The majority (>90%) of cells in EBs were ESCM that acquired almost the same structural and functional properties as typical CMs.

Conclusions The present study demonstrates the cardiomyogenic differentiation of ESCs can be efficiently controlled by co-cultured with native CMs, and this may lead to a practicable cocktail approach to generate ESC-derived CMs for stem cell-based regenerative medicine.

e0231 POLYMER-FREE SIROLIMUS AND PROBUCOL-ELUTING STENT FOR RENAL ARTERY: AN INITIAL EXPERIENCE IN SWINE

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Objective Bare metal stent (BMS) implantation can resolve renal artery stenosis successfully, but in-stent restenosis does occur, especially in small diameter renal arteries (<5.0 mm). The aim of this study is to test whether a newly designed polymer-free sirolimus and probucol-eluting stent (SPES) can inhibit neointimal hyperplasia of renal artery in swine.

Methods 26 stents (18 SPES and 18 BMS) were implanted in 36 renal arteries of 18 animals. During every procedure, a SPES and a BMS were randomised to the right or left renal artery. Seven animals were sacrificed after 90 days, and 11 after 180 days.

Results Histomorphometric analysis was performed. After 90 days, minimal lumen area, neointimal area, score of inflammation and score of endothelialisation were not significantly different between BMS and SPES. After 180 days, minimal lumen area was not significantly different between BMS and SPES (6.55±2.91 mm^2 vs 7.32±1.99 mm^2, p=0.477), but neointimal area was significantly less in SPES vessels than in BMS vessels (3.07±0.83 mm^2 vs 4.47±1.23 mm^2, p=0.005). Score of inflammation and score of endothelialisation were not significantly different between BMS and SPES after 180 days.

Conclusion SPES can successfully inhibit neointimal hyperplasia of renal artery in swine. At the same time, inflammation and endothelialisation in SPES vessels were similar to BMS vessels.

e0232 THE CHANGE OF VENTRICULAR INA AT DIFFERENT TIME OF SIMULATED ISCHAEMIA AND THE EFFECT OF ATORVASTATIN

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Objective To observe time dependent effects of simulated ischaemia on transient sodium currents (I_{Na}) of rat left ventricular myocytes, and the effects of atorvastatin on ischaemia I_{Na}.

Methods 30 Wistar rats were used for isolating left ventricular myocytes, which were randomly divided into two groups: ischaemia group (normal → simulated ischaemia) and statin group (normal → simulated ischaemia with 5 μmol/l atorvastatin). I_{Na} were recorded in normal condition (for control) by whole-cell patchclamp. Then in simulated ischaemia condition, I_{Na} were recorded from 3 to 21 min, monitored normalised peak currents every 2 min, and compared gate parameters between normal and simulated ischaemia (3 min) condition.

Results a. Normalised currents (at −40 mV), in ischaemia group, compared with normal (0.95±0.04), the currents in simulated ischaemia were increased to peak at 3 min (1.15±0.08, p 0.05, respectively), and decreased at 21 min (0.56±0.13, p 0.05). b. Gate parameters, from normal to simulated ischaemia condition at 3 min, membrane potential at 50% maximal activation ($V_{1/2,a}$), offsetting of activation curve (K_a), membrane potential at 50% maximal inactivation ($V_{1/2,i}$) and deinactivation constant (τ) were decreased ($p<0.01$, respectively) in ischaemia group, but offsetting of inactivation curve (K_i) were not changed; compared between two groups, K_i of statin group were decreased ($p<0.05$) and the decrease of τ value in statin group were less than ischaemia group ($p<0.05$).

Conclusions The effects of simulated ischaemia on I_{Na} are time dependent, while I_{Na} is transient increased at 3 min, and atorvastatin can depress this process.

e0233 LATE GADOLINIUM-ENHANCED MRI IN RESTRICTIVE CARDIOMYOPATHY

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Objective To evaluate the diagnostic value of MRI in combination of late gadolinium-enhanced imaging in the identification of restrictive cardiomyopathy (RCM).

Methods 116 patients with RCM underwent ECG, x-ray, Echocardiography and MRI. The final diagnosis was made on comprehensive evaluation in consideration of patients' history, clinical symptom and sign, imaging modalities. All patients had objective evidence of impaired cardiac filling and were referred to rule out pericardial thickening. Five histologically proven cases with RCM included heart transplantation in four patients with RCM, endomyocardial biopsy in one patient with RCM. Fifty-five normal subjects were used for reference. All patients were divided into two groups according to contrast-enhanced MRI: RCM with delayed enhancement (RCM with DE, n=35) and RCM without delayed enhancement (RCM without DE, n=81). Quantitative measurement of bi-atrial and bi-ventricular size, ventricular septal and left free wall thickness were done. A paired t-test was used for statistic analysis and a p value of less than 0.05 was considered significant. Qualitative assessment of segmental wall motion, in this present study.

Results The parameters, such as bi-atrial size, right ventricular diastolic diameter (RVDD), ventricular septal and left free wall

thickness were significantly larger in 116 patients with RCM than in normal subjects ($p<0.05$). However, there were no statistical differences between the two groups in left ventricular diastolic diameter (LVDD). Visual observation showed that mild mitral regurgitation (43%), moderate mitral regurgitation (21%), mild tricuspid regurgitation (28%) and severe tricuspid regurgitation (40%) were noted, respectively. 35 RCM with DE was further divided into diffuse and segmental enhancement. RCM with diffuse delayed enhancement was 15 cases, of which 12 cases showed powdery enhancement, and three showed petaline enhancement. Three cases with powdery enhancement were histologically proven as myocardial amyloidosis. RCM with segmental enhancement was 20 cases. Ventricular septum was the most vulnerable segment. Six cases presented subendocardial enhancement that corresponded to apical obliteration, of which one case was confirmed as hypereosinophilia with use of marrow examination. The other 14 cases didn't present any regular enhancement. 81 RCM without DE, of which histologically proven non-specific findings were in two cases, had marked bi-atrial dilation, near-normal ventricular chambers and near-normal ventricular thickness.

Conclusions MRI is an excellent imaging modality to identify restrictive cardiomyopathy. Primary RCM presents marked bi-atrial dilation with nonhypertrophied and nondilated ventricles. Diffuse left ventricular thickening associated with powdery enhancement indicates myocardial amyloidosis. Apical obliteration associated with subendocardial enhancement corresponds to endomyocardial fibrosis.

e0234 THE EFFECT OF LUTEOLIN ON H_2O_2-INDUCED VASCULAR SMOOTH MUSCLE CELL PROLIFERATION AND MIGRATION

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VSMCs Migration and proliferation are the critical pathological processes of various cardiovascular disease, such as atherosclerosis. Luteolin is a kind of flavonoids naturally occurring in many vegetables, fruits and medical plant. In this study, we investigated the effect of luteolin on the proliferation and migration of rat vascular smooth muscle cells (VSMCs) which were stimulated by H_2O_2, and a primary discussion was given to its mechanism of action. The present study demonstrated that Luteolin (12.5 to 50 μM) showed a particularly concentration-dependent inhibition effect on H_2O_2-elicited VSMCs proliferation and migration by MTT and Transwell assay respectively. In further research, we originally explored the function of Luteolin in blocking H_2O_2-triggered Src and Akt signalling pathways. The activation of Src, PDK1, Akt (308), Akt (473) of Luteolin group was significant lower than that of H_2O_2 group. These findings strongly suggested that Luteolin suppressed H_2O_2-directed migration and proliferation in VSMCs by inactivating Src and Akt pathways which participate in VSMCs migration and proliferation.

e0235 STUDY ON THE PRIMARY AND SECONDARY PROTEIN STRUCTURE OF ISCHAEMIA MODIFIED ALBUMIN

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Objective Ischaemia-modified albumin (IMA) has been demonstrated to be a biomarker of ischaemia associated with myocardial and skeletal muscle ischaemia, pulmonary embolism and stroke. However there is limited information on the formation mechanism of IMA. The aim of the study was to investigate the primary and secondary protein structure of IMA.

Methods This study included 29 acute coronary patients (IMA level>0.8 absorbance units) and 22 healthy controls (IMA level <0.5 absorbance units). Serum IMA was purified by salting out and ion exchange chromatography. We also chose 21 human albumin standard. The structures of purified protein and albumin standard were analysed by mass spectrometry, N-terminal sequencings and circular dichroism (CD) spectra measurement.

Results Protein sequences showed that the first 10 N-terminal amino acid residues of IMA were identical with those of albumin in healthy persons. The result of CD spectra measurement revealed that the average percentages of α-helixes and random coil decreased and the average percentages of β-turn and β pleated sheets increased a bit in ACS patients, but there is no significant difference between groups.

Conclusion Compared with the normal human albumin, no changes take place in the N-terminal protein sequence of IMA, and the secondary structure of IMA was also not significantly changed. Increasing percentage of β-turn and β pleated sheets in IMA may correlate with its formation mechanism.

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e0236 A REPERFUSION MODEL IN AMI RABBITS

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Objective To Explore the feasibility of establishing the reperfusion model on AMI rabbit by the method of obstructing and releasing the Left Anterior Ventricular Branch (LAVB) of left circumflex coronary artery (LCX).

Methods A total of 24 healthy Japanese albino rabbits of both sex were used in this study. Rabbits were randomly divided into 3 groups: ischaemia-reperfusion group (IR group, 8), ischaemia-noperfusion group (AMI group, 8), sham group (sham, 8). After preconditioning the myocardium twice by obstructing the blood flow for 5 min, we obstructed the flow of LAVB in IR group for 60 min, and then released it to be reperfusion. In AMI group we obstructed the flow permanently by ligating LAVB. And in sham group we only threaded but did not obstruct the flow. Then we killed them 3 days later. Venous blood was gathered. The levels of cTNI, CK and CK-MB were assayed at pre-reperfusion (baseline) and post-reperfusion period (4-h, 8-h, 12-h, 24-h, 48-h and 72-h after being reperfusion). The summation changes of ST segment elevation were observed in leads II, III, avF by ECG. Histopathology of myocardia, Evan's Blue and TTC dyeing were taken into notice. STATA8.0 software pack was used for data analysis.

Results The results come out that the ST segment all elevated in each group after LAVB was obstructed. In IR group the ST segment lowered more than 50% within 120 min after releasing the artery. This phenomenon had not appeared in other two groups. Compared to baseline, the cTNI, CK and CK-MB were all raised in IR group, and the peak value antedisplaced to 8 h, 12 h and 10 h. These three factors were all raised but no antedisplacement of the enzyme peak in AMI group. In sham group the raising of the three factors was slight, and no antedisplacement of the enzyme peak either. The experiment of rabbits in IR and AMI groups were consistent with the AMI diagnostic criteria in AMI Guideline of diagnosis and therapy established by Cardiac Disease branch of Chinese Medical