PTTG-2 and PTTG-3 expressions more dramatically decreased in hepatogenic differentiation of PDSCs. Otherwise, PTTGs expressions were increased in PDSCs transducing human TERT gene using lentiviral gene delivery system. Interestingly, the expression of PTTG2 was shown to increase in late passage of PDSCs. Down-regulation of PTTG1 and PTTG2 in PDSCs by siRNA inhibited self-renewal activity through decreased p53 expression. Taken together, the dynamic expressions of PTTGs in PDSCs regulate self-renewal as well as potential for differentiations. Therefore, these results not only further our understanding of the potential roles of PTTGs in PDSCs, but also contribute to the foundation for a potential new treatment strategy of cell therapy.

27.P09
Preparation of decellularized tissues: The importance of source animal age and thoroughness of decellularization upon the remodeling outcome

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Extracellular matrix (ECM) derived from decellularized tissues is increasingly being used for the reconstruction of injured tissues. Despite known variations in tissue remodeling outcomes, there is a paucity of quantitative studies which relate the source tissue properties or method of preparation to the remodeling outcome. The objectives of the present study were to evaluate: 1) the effects of source animal (pig) age (3, 12, 26 or >52 weeks), and 2) the effects of ineffective decellularization of source tissue (small intestine (SIS)) upon the in-vivo remodeling response. ECM strength, thickness and degradation resistance all increased with age. SIS-ECM from younger animals promoted progenitor cell chemotaxis while SIS-ECM from >52 week old animals promoted cell proliferation. More aggressive decellularization promoted a shift in macrophage phenotype from M1 to M2. Increasing SIS-ECM age was associated with an increasingly M1 host response and less constructive tissue remodeling. SIS-ECM from younger animals promoted the formation of new, innervated and vascularized, skeletal muscle tissue while SIS-ECM from old animals formed dense collagenous tissue in a rat model of abdominal wall reconstruction. It is clear that the age of the source animal and extent of decellularization are important determinants of a constructive tissue remodeling outcome.

27.P10
Optimization of physical tissue decellularization methods. A histological study

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Introduction: Decellularization consists on cell elimination without mechanical disruption of the extracellular matrix (ECM). The aim of this work is to evaluate two physical methods of tissue decellularization based on ultraviolet light (UV) and ultrasounds (US) exposition.
Methods: Mouse tissues were excised and washed in PBS. UV or US exposition during 10, 20 or 30 minutes were used as decellularization methods, with agitation. Histological analyses were performed by using 4',6-diamidino-2-phenylindole (DAPI), Gomori reticulin and Picrosirius staining. Statistical analysis was carried out by using student's t-test.
Results: Results of DAPI assay revealed that both methods were able to remove most of the cells from the tissues (95.10% for US and 96.87% for UV). Picrosirius stain demonstrated that US was able to preserve more efficiently the ECM collagen fibers (84.98%) than UV (73.46%) (p=0.000). Gomori reticulin staining showed that reticular fibers were better preserved using US (100%) than UV (83.54%) (p=0.000). No significant differences were found for different times (10, 20 and 30 minutes) for US or UV techniques.
Conclusion: US and UV techniques demonstrated to be efficient decellularization agents. The use of US seems to preserve more adequately the fibers of the ECM and should therefore be preferentially used in tissue engineering.
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27.P11
Preparation of biological tissue-polymer complex for percutaneous device

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Peritoneal dialysis is usually executed by implanting the flexible catheter directly the skin and peritoneal cavity. However, the problem with the flexible catheter is that the compatibility of the polymer and the skin tissue is too low. This often causes the down-growth of the epithelial cells which eventually causes the infection or even avulsion of the device. We tried to overcome this problem by preparing a percutaneous device based of a 'biological tissue-polymer complex', which possesses tissue compatibility and maintainability of the flexible catheter within the device at the same time. For the base material, the decellularized dermis was prepared by high hydrostatic pressure (HHP) method. By soaking the methyl methacrylate (MMA) monomer, benzoyl peroxide and N,N-dimethyl-p-toluidine into the decellularized dermis, we polymerized MMA at room temperature to obtain a dermis-poly(methyl methacrylate) (PMMA) complex. Scanning electron microscope images showed that PMMA formed a stable complex with the collagen fiber within the dermis. This complex induced the increase in the mechanical strength upon compression. It was possible to control the polymerization area limiting the central part of the decellularized dermis. So we prepared a tissue-polymer complex with the flexible catheter firmly held by the stiff PMMA in the middle of the dermis. We believe this complex is a promising material for percutaneous device which can be brought to the clinics in the near future.

27.P12
Marine sponges as natural scaffolds: decellularization by supercritical fluid technology and cellularization with osteoblasts for tissue engineering applications

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Marine sponges possess remarkable structures, with highly interconnected pores, making them excellent candidates as nature made scaffolds with potential use in tissue engineering strategies. In addition they also exhibit interesting high swelling degree and a skeleton composed mainly of collagen fibres, in some species with embedded siliceous or carbonate spicules. In the present study, several marine sponges (Dysidea avara, Chondrosia remiformis, Axinella damicornis,

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Petrosia ficiformis, Agelas oroides, Sarcotragus spinosulus, and Psammocinia sp.) were decellularized by supercritical fluids, for further use as natural scaffolds for osteoblasts. Supercritical fluid extraction was performed at 200 bar and 40 °C for 6 hours, in the presence of ethanol (40%), which was used as a co-solvent. The morphology of sponges, namely porosity interconnectivity and mean pore size, was analysed by SEM and micro-CT. To evaluate decellularization, sponges before and after treatment were analysed in sections stained with Hematoxylin-Eosin. Cellularization experiments were performed in S. spinosulus, using Saos-2 cell line. Cells were seeded on the sponge, with further evaluation of cell morphology by SEM, cell viability by Live/dead cell staining together with phalloidin.and cell proliferation by DNA quantification.The results of in vitro tests demonstrate the success of the proposed methodology for the production of natural scaffolds from marine sponges, namely for Sarcotragus spinosulus.

27.P13
Effect of urinary bladder matrix on the in vitro performances of human mesenchymal stem cells

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Biomaterials, including natural extracellular matrix (ECM), have been proposed as scaffold for tissue engineering. It is important to consider that tissue morphogenesis is influenced by the interaction of cells with the complex architecture/composition of natural ECM. Simple polymers such as collagen, gelatine and fibronectin, have been used for cell seeding/entrapping and in vivo implantation, providing mechanical support to cells, but do not adequately mimicking the interactions between cells and ECM. On the contrary, ECM-based biomaterials can provide a native framework for cell adhesion, at the site of a tissue deficit, allowing local cells to migrate into the matrix, adhere and differentiate. The use of urinary bladder matrix (UBM) as scaffold material can provide the structural support and dynamic exchange signals to local cells leading to tissue infill. The major constituents of UBM are collagen, glycoproteins, glycosaminoglycans and various growth factors. This study describes the ability of UBM based scaffolds to support the adhesion, proliferation, 3-D colonization and osteogenic differentiation of human mesenchymal stem cells form human umbilical Wharton's Jelly (hWJMSCs), when compared to traditional two-dimensional (2-D)cultures. Our data strongly suggest the combination between UBM and hWJMSCs represents a promising approach to the use of natural and biocompatible ECM-based biomaterials for cell culture of mesenchymal stem cells.

27.P14
Extracellular matrix derived from kidney regulates the growth and metabolism of kidney stem cell with regional specificity

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Extracellular matrix (ECM) provides cells with an ideal scaffold with specific cues that mediate cell function. Stem cells are known to reside on specialized ECM niches where they remain quiescent until needed, such as stem cells in the papilla region of the kidney. Currently it is not possible to re-create the complex environment of the kidney using synthetic materials. Therefore, our objective was to use the native ECM to investigate if there were region specific effects on the growth of kidney stem cells (KSCs) and mouse mesenchymal stem cells (MSCs). We derived hydrogels from three kidney regions: cortex, medulla and papilla. MSCs were purchased and KSCs were isolated from the renal papilla of mouse kidneys. Porcine kidneys were sectioned into: cortex, medulla and papilla regions. Each region was decellularized with 0.02% trypsin, 3% Tween, 4% deoxycholic acid, and 0.1% peracetic acid solutions followed by enzymatic digestion. Cells were cultured on the hydrogels or in media supplemented with digested ECM. Metabolic activity, image analysis and DNA quantification were performed. KSCs cultured in the presence of papilla ECM showed higher metabolic activity and lower DNA content when compared to the other ECMs, an effect not observed using MSCs. These results suggest that the hydrogels derived from the native kidney ECM stimulate KSCs but not MSCs. Region-specific ECM may thus provide an optimal substrate for cultivation and delivery of stem cells and their derivatives.

27.P15
Optimizing decellularization of porcine kidneys for whole organ bioengineering

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Kidney transplantation is currently the only treatment that can restore renal function, however, donor supply meets less than one-fifth of demand. Regenerative medicine offers an alternative through whole organ decellularization and recellularization. The goal of this study was to evaluate detergents for effective decellularization of porcine kidneys and subsequent sterilization methods. Kidneys from Yorkshire pigs were obtained then decellularized with various detergents (SDS, SDC, Triton X-100, NP-40 or Tween-20) followed by rinsing. Resultant tissue samples were quantified for residual DNA and sGAGs. Whole acellular scaffolds were either gamma-sterilized or perfused with chemical sterilants with biopsies placed in growth medium to determine contamination or processed for histology. Only SDS was capable of removing cellular material as demonstrated by a lack of nuclear staining and a 99% reduction in DNA levels compared with fresh tissue. SporGon treatment or gamma-irradiation ≥1MRad sterilized whole kidney scaffolds, however, this resulted in morphological changes to acellular tissue. Sulfated GAGs were not significantly reduced following 1MRad irradiation. These studies show that SDS detergent perfusion removes all cellular material from porcine kidneys. 1MRad gamma irradiation provided a sterile scaffold which demonstrated human cell biocompatibility. This study represents a significant step towards a transplantable graft from porcine-derived acellular whole kidneys.

27.P16
A critical evaluation of kidney extracellular matrix after perfusion decellularization as a structural basis for renal tissue engineering

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The kidney is the most frequently transplanted organ, yet few studies have focused on kidney tissue engineering. We compared three decellularization protocols in rat kidneys to create extracellular matrix (ECM) scaffolds for tissue engineering applications. We have further analyzed these scaffolds using rigorous methods for: 1) retention of critical matrix structures, 2) efficient removal of cells and 3) presence of structural and growth factor proteins. Three different protocols were used: Triton only (P1), Triton and SDS (P2) and Triton and Trypsin (P3). Hematoxylin & eosin (H&E) staining and scanning electron microscopy

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