Repair of substantial cranial defects in adults and children may be compromised due to limitations in donor bone stocks for autologous grafts. We evaluated the capability of autologous adipose derived mesenchymal stem cells (ADSC) in combination with Poly-Lactic Acid (PLA) scaffolds to regenerate bone in a critical-sized skull defect. Thirty adult New Zealand White rabbits were divided into 6 groups of 5 animals each consisting of: 1) PLA alone (control); 2) Fibronectin-coated PLA; 3) PLA with ADSC; 4) Fibronectin-coated PLA with ADSC; 5) PLA with osteogenically induced ADSC (osADSC), and 6) Fibronectin coated PLA with osADSC. All the animals were sacrificed after 6 weeks. X-Ray, histology and histomorphometric analysis were performed in order to evaluate the new bone formation inside the PLA scaffold. Radiographically and histomorphopmetrically, the groups in which the PLA was not fibronectin coated showed no bone formation in contrast to the fibronectin coated groups; the group treated with osteo-induced ADSC and fibronectin showed significantly more bone formation than the group treated with undifferentiated ADSC and the group treated without cells. These data indicate that the surface treatment with fibronectin permits cell attachment and survival within the scaffold, and that autologous, osteo-induced adipose-derived stem cells can regenerate bone if seeded into a surface-treated PLA scaffold.

(OP 14) Alginate Film as a Tissue Adhesion Barrier

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Adhesion formation is a well-known complication of abdominal surgery, which not only renders future operations more difficult but also is the most common cause of small bowel obstruction, female infertility, and chronic debilitating pain. In order to reduce post-surgical adhesion formation, physical barrier to isolate the traumatized tissue from surrounding organ was commonly used. It should have adhesiveness in injured site, biodegradibility, mechanical strength and flexibility for handling. Various natural and synthetic polymer sheets or membranes have been developed as non-absorbable or absorbable physical barriers, however, their loose contact with applied tissue needed for sutural fixation. It has been also reported that a variety of polymer solutions or gels such as hyaluronic acid, dextran, polyvinylpyrolidone, carboxymethyl cellulose and polyethylene glycol were used to prevent tissue adhesion. However, their easy washing out before the healing of injured tissues are still remained as a critical limtation.In this study, we prepared an alginate film to estimate its potential use as an abdominal adhesion barrier. We expected that the non-crosslinked alginate film can have good anti-tissue adhesion property, owing to its good mucoadhesiveness with injury site (inhibition of film migration without suturing) and low tissue affinity of slowly solubilized alginate in the body (prevention of tissue adhesion). The *in vivo* animal study using a rat model was carried to evaluate the anti-tissue adhesion effect of the prepared alginate film. It was also compared with those of crosslinked alginate film, alginate solution, and commercialized anti-adhesion membrane (InterceedTM).

(OP 15) Amniotic Fluid-Derived Stem Cells for Regenerative Medicine Therapies

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Cell sourcing for tissue engineering remains a major challenge for clinical translation of regenerative medicine therapies. Stem cells represent an alternative source of cells in instances where differentiated cells can not be expanded *in vitro*. Human amniotic fluid has been used in prenatal diagnosis for more than 70 years. It has been demonstrated to be a safe, reliable, and simple screening tool for a variety of developmental and genetic diseases. A subset of cells in amniotic fluid has been shown to have stem cell properties and express stem cell markers. Amniotic fluid stem (AFS) cells are capable of maintaining prolonged undifferentiated proliferation as well as of differentiating into multiple tissue types that encompass the three germ layers (mesenchymal, ectodermal and endodermal). These cells show a high self renewal capacity (>300 population doublings) and clonal growth capability, but unlike embryonic stem cells, they do not require a feeder layer and do not form teratomas when injected *in vivo*. These findings indicate that amniotic fluid may have increased utility than as a diagnostic tool and may become a preferred therapeutic cell source for a multitude of congenital and adult disorders.

(OP 16) An Oscillatory, Perfused Bioreactor for Cell and Tissue Culture

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Bioreactors can: (i) maintain viability of harvested cells prior to transplant, (ii) seed cells onto porous 3D scaffolds, and (iii) enhance tissue regeneration, by providing physiologic culture conditions and convective-diffusive mass transport. A single device complying with these specifications is an oscillating perfused bioreactor wherein 1 to 12 gas-permeable chambers are stacked on an incubator–compatible base that provides bi-directional flow of culture medium at a controllable rate (~0.40 to 5.0 mL/min; ~0.20 to 2.7 mm/s). This bioreactor was validated for cells cultured in suspension and on 3D scaffolds (hyaluronic acid-based non-wovens or sponges; collagen sponges). Suspension culture was demonstrated by inoculating primary heart cells into chambers. Bioreactors yielded higher ($48 \pm 5\%$) cell viabilities than orbitally mixed or spinner flask controls on culture day 8. Perfusion seeding and culture was demonstrated by inoculating chondrocytes into chambers containing porous scaffolds. Bioreactors yielded more spatially uniform cell distributions and higher DNA/construct ($p < 0.001$) than static controls on day 4, and enchanced cartilaginous matrix production to yield thicker contructs than static or spinner flask controls ($p < 0.001$) on day 14. Perfusion culture after seeding was demonstrated after hydrogel entrapment of heart cells within porous scaffolds. Bioreactors yielded more aerobic cultures (lactate produced/glucose consumed, $p < 0.05$), higher cell viability ($p < 0.01$), and lower apoptosis ($p < 0.01$) than static controls. Moreover, heart cell elongation was observed only in bioreactors. In conclusion, an oscillatory perfused bioreactor

preserved viability of freshly harvested cells and enhanced *in vitro* regeneration of tissue engineered cartilage and myocardium in the context of a modular, scalable, customizable system.

(OP 17) Application of an Autologous Mixture of Platelet Rich Plasma, Endothelial Progenitor Cells and Keratinocytes Promotes Matrix organisation, Neo-vascularisation and Reepithelialisation in Porcine Full Thickness Wounds.

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In search for an autologous vascularised skin construct, we treated full thickness wounds (FTWs) with a mixture of basal cell keratinocytes (KCs) and endothelial progenitor cells (EPCs) embedded in platelet rich plasma (PRP). This autologous gel functions as a guiding scaffold and a source of growth stimulating factors.

We cultivated autologous basal KCs in low serum conditions and expanded autologous EPCs from the internal jugular vein. FTWs ($n = 55$) were created on the back of in total 4 pigs, covered with transparent flexible wound chambers and randomly assigned to: 1. Saline; 2. PRP; 3. PRP + KCs; 4. PRP + EPCs; 5. PRP + KCs + EPCs. All wounds were biopsied (day 8) to measure neovascularisation (lectin BS-I, aSMA and MT1-MMP), matrix deposition (fibronectin and collagen I/III) and reepithelialisation. Wound fluids, aspirated daily, were analysed for protein expression.

All EPC treated wounds showed 6–8 times more vascular structures compared to saline ($p < 0.001$). Addition of PRP to EPCs further improved neo-vascularisation, confirmed by higher lectin, αSMA and MT1-MMP. All PRP treated groups showed higher collagen I/III deposition ($p < 0.05$) and a higher fibronectin content ($p < 0.001$). PRP treated wounds exhibited higher concentrations of pro-angiogenic growth factors in wound fluid samples. Application of PRP+KCs resulted in highest reepithelialisation rates compared to saline ($p < 0.001$).

In this porcine FTW model, PRP acts as a supportive biomatrix, creating a more developed vascular network, improving extracellular matrix organisation and leading to accelerated reepithelialisation. Addition of EPCs further enhanced the pro-angiogenic properties of this matrix.

We currently use this template in our 'MilleFeuille' autologous tissue engineering protocols to create laminated vascularised tissue layers.

(OP 18) Application of Fluorescence Techniques to the Study of Protein Adsorption and Packing on Biomaterial Surfaces

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The ways proteins compete for the surface of biomaterials and change conformation are believed to be important for the host response to implants. It is possible to elucidate information on packing and any induced conformational change by making use of different fluorescence techniques on fluorescently labelled proteins. Employing probe-probe resonance energy transfer (RET) allows inter and intra protein interactions to be distinguished. Homo resonance energy transfer (hRET) avoids many problems with having two different probes and means that labelling and subsequent purification can be done in one step.

In this study we made use of both steady state and time-resolved fluorescence techniques and imaging to study FITC (fluorescein isothiocyanate) tagged BSA (bovine serum albumin) adsorption to various (fluorescent) polymeric biomaterials (poly-caprolactone, starch polycaprolactone and starch ethylenevinylalcohol) using titania coated glass as a control. With the combination of both steady state anisotropy and lifetime methods applied on different dilutions (labelled:unlabelled and label:protein) of FITC-BSA differences in packing on the surfaces was determined. The anisotropy data indicated inter protein hRET, more clearly on the control. Dilution of over-labelled proteins in unlabelled seems to be easiest way to make sure the signal is from intraprotein interactions, but polymer autofluorescence requires addressing.

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(OP 19) Articular Chondrocytes Culturing Conditions: Optimization and Drawbacks for Cartilage Resurfacing Attempts

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Cell-based cartilage resurfacing requires the *ex-vivo* expansion of autologous articular chondrocytes (ACI) and the maintenance of cell differentiation potentials. Culture conditions have been devised to minimise cellular phenotypic changes, but their clinical applications have to cope with outcomes-undermining aspects: a) cell-source biopsies display different physio-pathological conditions; b) defined growth media composition may not be optimal to establish cartilage repair. We hypothesized that non-frank osteoarthitic (OA) chondrocytes could affect ACI outcomes. To this purpose human cartilage specimens were prepared from 65–75 year-old donors. Normal and early grade I-OA samples were examined histologically and used to derive primary cell cultures. OA chondrocytes showed immunopositivity to MMP-3, negativity to type II collagen and reduced matrix components in micromass cultures. We showed that Sox9, a cartilage-specific transactivator, is prevented from binding its responsive elements on the promoter of the Cartilage Oligomeric Matrix Protein (COMP) gene, coding for an extracellular protein. Clearly, a reduced Sox9 availability minimizes the cell chondrogenic potential. To improve it, OA