to the magnet in all tested thicknesses. Examination of the magnet’s mean force of attraction at thickness A, B, and C was 0.55N, 0.16N, and 0.04N, respectively, representing a statistically significant difference between attraction at A versus C (p<0.001).

CONCLUSION: Industry standard magnets lose significant magnetic attraction with increased distance from TE ports. Accurate port localization is important to prevent iatrogenic injury to the tissue expander during the expansion process. This benchtop study demonstrates that the Port-Findr is significantly more accurate at locating the center of the port, than a dangle magnet, through all simulated thicknesses tested. Furthermore, precision, represented by standard deviation, was better in tests using the PortFindr. More accurate and precise localization of subcutaneous ports may help reduce complications.

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A Novel Skin Whole Organ Culture Technique Maintains In Vivo Cellular Characteristics and Population Profiles

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PURPOSE: In reconstructive surgery, large areas of tissue loss represent a major surgical obstacle. Where split-thickness skin grafting and flap transfers fail, cell-based treatments represent a promising therapeutic option. Currently, cell therapies are limited to transplants from non-autologous donors, or expanded isolated skin components (e.g., keratinocytes only). However, literature suggests that keratinocytes and fibroblasts act synergistically to restore functional tissue.1 Typical culture conditions poorly mimic in vivo conditions, and skin cells change dramatically after plating.2 Thus, there is a demand for techniques to expand multiple autologous cell types without fundamentally altering cell behavior. Here, we describe methods for the ex vivo culture of skin cells that allow for efficient expansion while maintaining in vivo cell characteristics.

METHODS: Adult mouse skin was harvested and sterilized using gradated iodine solutions. Tissue was chopped with sterile scissors followed by digestion with 0.5 mg/mL Liberase™ DL (Roche). Cells were grown in DMEM/F12 with 10% fetal bovine serum and 1% penicillin-streptomycin, on polystyrene coated with 0.1% gelatin (EmbryoMax) or in 3D collagen hydrogels of varying stiffness. Morphology was assessed via imaging and analysis using Photoshop CS6 (Adobe). Relative cell populations were quantified using fluorescence-activated cell sorting (FACS). Isolation of Engrailed-positive fibroblasts (EPFs), the dermal fibroblast population responsible for wound healing (collagen deposition), was achieved by FACS of cells from En1Cre;R26mTmG mice.

RESULTS: Skin cells grown via whole organ culture on gelatin-coated polystyrene had no significant change in resident cell population density over multiple passages (2–4% fibroblasts; of non-fibroblasts, 50–60% blood cells; remainder keratinocytes; P>0.05). Upon isolation from whole organ culture, fibroblasts of a single population (EPFs) demonstrated expansion by over 20-fold in two passages. With traditional culture methods, fibroblasts demonstrate increased cell size over repeated passages; in contrast, these phenotypic shifts in EPFs were rescued by culturing in 3D hydrogels or on gelatin-coated polystyrene. Specifically, EPFs grown on gelatin-coated polystyrene demonstrated no significant change in cell size from passage (P)1 to P3 (average fold change=0.879, n=3 biological replicates, all P>0.05).

CONCLUSION: By removing many of the artificial selection pressures that cells experience in culture, we accomplished efficient ex vivo expansion of in vivo-like skin cells. Specifically, by employing whole organ culture rather than culturing cells in isolation, nonselective media, and 3D hydrogels to mimic in vivo mechanical tensions, cells retained their in vivo morphology and population densities. Autologous cell-based therapies hold increasing promise for complex reconstructive surgery, and our results signify a therapeutically relevant advancement that may enable improved cosmesis and functionality of transplanted skin organs. With similar expansion of human skin, a 4mm punch biopsy alone could yield the equivalent of over 250 mm² of skin for transplantation. In the future, we will verify our technique using epigenetic studies and machine learning-based assessment of cell morphology,
and will use our technique to create 3D organoids capable of transplantation.

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A Novel Technique for Tissue Engineering Periosteum Using Three-Dimensional Bioprinting

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BACKGROUND: Periosteum plays a vital role in bone repair by housing a population of cells with remarkable regenerative capacity, including osteoblasts and mesenchymal stem cells. Periosteal flaps are often used by reconstructive surgeons to promote healing of bone defects caused by trauma, congenital deformities, or tumor resection. However, the utility of periosteal flaps is limited by donor-site availability and morbidity. This study presents a unique method for periosteum tissue engineering in which a three-dimensional (3D) bioprinter was used to print a precise pattern of periosteal derived cells (PDCs) onto collagen scaffolds.

METHODS: PDCs were isolated from the periosteum of bovine femurs and expanded in culture. PDCs were then mixed with alginate gel to create a bio-ink, which was printed in three different experimental groups: bio-ink alone; bio-ink printed on a type I collagen (COL1) scaffold; and bio-ink printed on a type II collagen (COL2) scaffold. To maximize the viability of the PDCs, the bio-ink was printed in a precise lattice pattern that was created using computer-aided design (CAD) software. PDCs were also cultured in monolayer (no alginate, no collagen) to serve as a control group. All groups were incubated in culture media and evaluated at one and two week time points. Live/Dead staining was used to assess cell viability. Polymerase chain reaction (PCR) was used to quantify gene expression and assess osteogenic differentiation.

RESULTS: On gross examination, the COL1 and COL2 scaffold groups maintained greater structural integrity than the bio-ink only group. Live/Dead imaging showed high viability of cells at one and two weeks in all experimental groups. PCR results demonstrated an increase in gene expression of the osteogenic differentiation markers osteocalcin (OCN) and alkaline phosphate (ALP) in all treatment groups relative to the monolayer control group. OCN expression was most significant in the COL1 group. PCR also showed an increase in COL2 gene expression in all treatment groups, but most significantly in the COL2 scaffold group.

CONCLUSION: The results presented here support a novel method for using 3D bioprinting to engineer periosteum constructs. The COL1 and COL2 scaffolds promoted cell viability and structural stability. Increases in OCN and ALP gene expression suggest the PDCs were undergoing osteogenic differentiation, with the COL1 scaffold being most supportive of this phenotype. The increase in COL2 gene expression, a chondrogenic marker, suggest some of the PDCs may be undergoing early chondrogenic differentiation before endochondral ossification into bone. In vivo studies are currently underway to assess the capacity of this tissue engineered periosteum to induce bone repair in an animal model.

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Harnessing Mechanical Cues to Enhance Cellular Migration in a Novel Tissue Engineered Dermal Substitute

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