several factors including: age, race, neoadjuvant therapy, grade of differentiation, chemotherapy, radiation therapy, and type of insurance. A logistic regression model was developed to identify factors predictive of reconstruction.

**RESULTS:** From 2000–2017, 5,890 patients met eligibility criteria and were enrolled in this study. Mean age was 57.6 years and the participants were predominantly Caucasian (87%). Out of 5,910 participants, 3,831 (64.8%) underwent breast reconstruction. As shown in Table 1, significant variations in rates of breast reconstruction were found across patient age group (p = 0.005), grade of cancer (p < 0.0001), type of insurance (p = 0.001), use of chemotherapy (p = 0.002), and use of radiation (p = 0.0002). Notably no significant rate of breast reconstruction was found across race (p = 0.537) or use of neoadjuvant therapy (p = 0.665). Controlling for these factors, logistic regression analysis revealed that undifferentiated grade of cancer (OR: 0.67, p = 0.003) and patients with Medicare insurance (OR: 0.41, p < 0.0001) were the most predictive clinical factors for not undergoing reconstruction, whereas patients who received chemotherapy were actually more likely to undergo breast reconstruction. Interestingly, while rates of breast reconstruction after radiation treatment were high (62%), after controlling for other factors, radiation also became a negative predictor of breast reconstruction after mastectomy (OR 0.72, p < 0.0001)

**CONCLUSION:** Independent of treatment modality, poor tumor grade seems to be an important factor in predicting breast reconstruction rates at our institution. Further work is necessary to elucidate why this relationship holds true for surgical treatment independent of more aggressive medical treatment of breast cancer.

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**Three-Dimensional Scaffold-Free Spheroids with Fibroblast/Macrophage Co-Culture for in vitro Fibrosis Modeling**

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**PURPOSE:** An *in vitro* model of fibrosis is critical for designing surgical implants with better biocompatibility or new anti-fibrotic drug development. However, traditional 2D monolayer culture-based models of fibrosis show little resemblance to the *in vivo* fibrogenesis process. To enable screening of exponentially larger numbers of materials and conditions than can be achieved by sub-acute animal testing, we have developed a 3D scaffold-free spheroid system with human fibroblasts (spherical micro-tissue), which is more physiologically similar to real tissue. In addition, hybrid spheroids consisted of human fibroblasts and macrophages have been fabricated to investigate direct fibroblast-macrophage interaction and communication during fibrogenesis.

**METHODS:** Spheroids were fabricated of 100% human fibroblasts with difference sizes (100, 200, 300, 400, 500µm in diameter) respectively. Immunofluorescent staining with collagen-1 and apoptosis marker (Caspase-3) as well as qPCR with fibrosis genes (collagen-1 and αSMA) was performed to compare with the 2D control to identify the optimal size of the spheroids for an effective fibrosis model. In addition, we isolated human peripheral blood derived monocytes to differentiate into macrophages that could be used to populate the spheroids. Macrophages were co-cultured with fibroblasts under different ratios (1:2, 1:4, 1:8, and 1:16) to identify the ideal ratio in hybrid spheroids. We performed immunofluorescent staining with macrophages marker (MAC387) and fibroblasts marker (ER-TR7) and qPCR to optimize the ratio of macrophages in the hybrid spheroids to optimize similarity to physiologic *in vivo* fibrogenesis.

**RESULTS:** Fibroblasts in spheroid had higher expression levels of fibrosis genes compared to the 2D monolayer control. The spheroid size of 200µm showed the highest viability, more homogenous collagen deposition and the highest expression level of fibrosis genes due to a lower expression level of proteinase-related genes such as MMP1, MMP2 and MMP7. Interestingly, hybrid spheroids at a ratio of 1:16 (macrophage:fibroblast) showed higher survival rate of macrophages and a remarkably higher expression level of fibrosis genes (collagen-1, collagen-3 and TGFβ) with a lower expression level of anti-fibrotic MMP7.

**CONCLUSIONS:** We have developed a 3D scaffold-free spheroid system as a more physiologically relevant fibrosis model *in vitro* for implant material and drug development and screening as compared to current methods. The inclusion of macrophages led to a significant change in fibroblast behavior. The size of the spheroids and the ratio of macrophages have been optimized regarding the resulting fibrosis profile. Future work will involve correlation of our
findings with the in vivo tissue response.

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AMD3100 (Plerixafor) As A Single-Dose Stem Cell Mobilizing Agent In Vascularized Composite Tissue Allograft (VCA) Transplantation In A Canine DLA-Mismatch Model

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PURPOSE: Vascularized Composite Allograft (VCA) transplantation is a clinical reality but limited by toxicities of chronic immunosuppression and rejection. Current clinical tolerance protocols rely on recipient conditioning and donor cell mobilization that limits use to living donor transplants. We sought to design a clinically relevant protocol applicable to cadaveric organs. We previously demonstrated that using AMD3100 (Plerixafor) as a single-dose agent for stem cell mobilization was successful in a DLA-haploidentical model. We wanted to increase clinical relevance by testing our existing non-myeloablative stem cell canine VCA transplant model to DLA-mismatched, unrelated canine donor-recipient pairs.

METHODS: Three DLA-mismatched, unrelated canine recipients [Group I] received conditioning with 450cGy TBI, AMD3100-mobilized donor stem cells + Bone Marrow (BM) infusion and simultaneous VCA transplantation with a short course of immunosuppression (Sirolimus: 28 days/MMF: 56 days/CSP: 70 days). Three DLA-mismatched, unrelated canine recipients [Group II] underwent a less intense conditioning regimen (350cGy TBI) but otherwise identical transplantation protocol. CD34+ hematopoietic progenitor cells were quantified via flow cytometry. Peripheral blood chimerism was evaluated by PCR techniques weekly. VCA graft survival was followed clinically and histologically.

RESULTS: All six canines tolerated the conditioning regimen. Stem cell engraftment and donor chimerism was seen in all dogs. Mean COBE apheresis count was 4.28x10^8 cells/kg and mean BM aspirate count was 0.81x10^8 cells/kg across both groups. Outcomes varied. No evidence of acute rejection was seen. Two dogs demonstrated signs of VCA rejection once off immunosuppression. GVHD (skin and/or liver) was seen in two dogs. Two dogs were lost post-operatively to the unexpected complication of intussusception while still seemingly tolerant to the VCA.

CONCLUSION: This study demonstrates proof of principle for AMD3100 as a single-dose stem cell mobilizing agent for a clinically relevant tolerance protocol in mismatched, unrelated donor-recipient pairs. Use of AMD3100 led to stem cell engraftment in all animals transplanted with no evidence of acute rejection in the VCA. AMD3100 use limited by thrombocytopenia in our previous studies continue to appear be resolved with the addition of BM Aspirate in this model. Continued experiments should allow for longer-term follow up in future canine recipients that should optimistically not experience bowel complications or GVHD.

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And at last, the Wound is Healed... or, is it?! In Search of an Objective Way to Predict the Recurrence of Diabetic Foot Ulcers

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PURPOSE: Diabetic foot ulcer (DFU) has a lifetime incidence of up to 34% among diabetics. Up to 49 million patients worldwide have a history of DFU which comprises the primary etiology for 75% of all amputations. The recurrence rate of DFUs is alarmingly high (40% within 1 year, and 65% within 5 years), with no reliable methods available to predict its occurrence. The current definition of “complete wound closure” relies on a visual assessment to determine “skin re-epithelialization without drainage”. However, many of such presumably closed wounds may still remain “functionally” open and deficient in skin barrier function, putting them at higher risk of recidivism. We hypothesize that impaired skin barrier function is an early indicator of DFU recurrence, and propose a non-invasive, point-of-care testing to measure this property based on trans-epidermal water loss (TEWL).