The unpredictable outcomes following autologous fat grafting are likely a multifactorial process caused in part to the fragile nature of adipocytes which makes them susceptible to trauma at the time of harvest and a high metabolic demand known of these cells, which may limit the longevity of any cells that do survive the harvest process.

In an effort to improve the outcomes following autologous fat grafting, our early research has focused on addressing these impediments to graft survival. Syringe- and needle-aspirated fat immediately following harvest is comprised of a mixture of live cells, dead cells, cell debris, and free oil (Figure 1, A). However, when this cell slurry was subjected to a novel purification regimen, live cells were singly isolated (Figure 1, B)—a cell suspension with a viability and purity of 97% and 94%, respectively, helping to reduce the load of dead tissue and inflammatory debris.3

To address the high metabolic demands of these cells, these purified adipocytes were then suspended in a resorbable 3-dimensional protein matrix (GFR Matrigel; BD Biosciences, San Jose, CA) to provide greater initial graft stability in hopes of offering more rapid revascularization and growth factor transfer. When grafts were prepared in this fashion and injected into mice, on tissue section at 10 days (Figure 2), the grafts lacked the widespread inflammatory cell infiltrate, necrosis, and vacuolization commonly seen in unpurified fat graft controls (Figure 3). More importantly, these tissue-engineered fat grafts showed a 3-fold improvement in volume maintenance.

The present study sought to better understand the role played by the resorbable matrix in this process. The hypothesis was that the matrix composition plays an important role in graft survival. To test this hypothesis, two different resorbable suspension matrices were compared head to head: PuraMatrix (3DM; Cambridge, MA)—a cross-linked peptide hydrogel made of inert amino acids—and GFR Matrigel—a conglomerate of 3 basement membrane proteins which more closely approximates native extracellular matrix.

METHODS
Twenty genetically identical age- and sex-matched B6/C57 mice were used as subjects. Each subject received five

Drs. Piasecki, Moreno, and Gutowski are from the Division of Plastic and Reconstructive Surgery, Department of Surgery, University of Wisconsin School of Medicine, Madison, WI.
grafts, each randomized to one of six positions on the mouse’s back. Two grafts were experimental (comprised of purified fat cells suspended in each of the two resorbable matrices). Each of the purified fat/matrix grafts contained statistically the same number of viable adipocytes per graft (Figure 4). Three control grafts were also placed: one comprised of unpurified fat alone (harvested with a syringe and 18-gauge needle) and one made up of each of the two matrices alone. At defined time points, external volumes and histology were performed.

RESULTS

Despite starting with only one-half to one-third as many viable adipocytes per graft when compared to unpurified fat graft controls (because of the large dilution factor of the matrix on the purified fat cells within each of the purified fat grafts; Figure 4), when graft volumes were plotted over time, purified fat/GFR Matrigel grafts statistically outperformed all other groups maintaining more than 80% (95% confidence interval [CI], 58.5–101.5) of their volumes at 3 months (Figure 5). Interestingly, purified fat/PuraMatrix grafts did not perform any better than unpurified fat graft controls, maintaining only 19.6% of starting fat volume (95% CI, 0–42.3).

When histologic sections were performed, profound differences were also observed. The relative presence of intact adipocytes at 3 months was markedly disparate between grafts groups (Figure 6), with purified fat/GFR...
Matrigel grafts 70.5% (95% CI, 68.4–73.6) comprised of intact fat cells, versus only 5% (95% CI, 3.8–7.2) and 7% (95% CI, 4.5–9.5) for unpurified fat graft controls and purified fat/PuraMatrix grafts, respectively (Figure 7). With respect to tissue fibrosis, the reverse was true (Figure 8), where unpurified fat graft controls and purified fat/PuraMatrix grafts were 96% (95% CI, 94.7–97.3) and 90.1% (95% CI, 86.8–93.4) comprised of fibrotic material (Figure 9). The relative contribution to graft volume of residual matrix was negligible (Figure 10), occupying only 6% (95% CI, 4.8–7.2) of purified fat/GFR Matrigel grafts at 3 months (Figure 11). Purified fat/GFR Matrigel grafts showed a statistically greater vessel density versus other groups at 3 months as well (Figure 12).

The fate of the matrix control grafts was complete resorption in all cases. However, these grafts did show differential resorption patterns, with GFR Matrigel resorbing slowly over 62 days (95% CI, 55–69), and PuraMatrix resorbing quickly over 10.3 days (95% CI, 5.2–15.4; Figure 13). Neither of these control graft types showed any sign of tissue ingrowth or vasculogenesis during their unceremonious resorption (Figure 14).

**DISCUSSION**

Autologous fat grafting can be an unpredictable enterprise, likely because of multiple factors. Currently, most clinicians employ some form of cellular purification regimen before graft administration. Although this is clearly an important aspect of graft performance optimization, a focus only on the cells, in a “cell-centric” approach, may be short sighted. These data suggest an important role of the pericellular, extracellular matrix proteins in grafted cell survival—for indeed, suspending...
the same number of viable cells in different protein matrices dramatically affected the macroscopic and microscopic performance of these grafts.

While a difference was clearly noted, what is not clear from this study is why. Critics of the study design might point to a hypothetically toxic effect of PuraMatrix on suspended cells. Although this cannot be ruled out entirely, this is unlikely. While PuraMatrix has not been studied with adult fat cells in this fashion before, its successful use has been reported in the cell biology literature as a plating and suspension matrix for a variety of cell types, all more fragile than adipocytes. Additionally, if there was a toxic effect of PuraMatrix on the purified fat cells, one would logically expect cell lysis and secondary inflammatory cell infiltrate, and this was not seen on tissue section at any time point.

The different temporal resorption patterns of the two matrices may have played a role as well. It has been suggested in the literature that more structurally stable fat grafts perform better. This may be analogous to a stable bolster atop a skin graft, which allows for efficient inosculation and capillary ingrowth by preventing movement and shear. However, in contrast to the skin graft, we as clinicians ask far more of fat grafts—they are placed not as a single sheet, but rather in greater bulk to fill a 3-dimensional space; contradictory to this is the fact that adipocytes require much faster revascularization than skin cells, with a baseline metabolic rate 2 to 3 times that of skeletal muscle. We ask more of them, but in standard practice today, offer them less. Perhaps a stable matrix of unclear ideal duration (in this study, > 10 days) would afford this. Such a theory supports clinically observed success as described by Coleman with multiple small aliquots injected into the local tissue stroma—the recipient tissue bed itself acting as a biologic bolster.

It is likely however, that the individual matrix components also play a complex metabolic role. It stands to reason that if PuraMatrix was truly inert, and lasted 10 days, that it offered at least adequate initial support. Despite this, graft performance was still dismal. GFR Matrigel’s 3 components (heparin sulfate, laminin, and type IV collagen) have potential biologic roles in cell survival: heparin sulfate is known to bind growth factors; laminin binds well to adipocytes. Perhaps a combination of additional stability and the facilitation of intercellular growth factor transfer would provide more rapid revascularization early on in graft take.
Two other points are worth addressing here. The first concerns the plane of administration. In this study, the fat grafts were placed in the subcutaneous layer. Most clinicians would agree that subcutaneous fat is not as vascularized as other tissues prompting some surgeons to inject smaller volume fat grafts into muscle or dermis. Certainly if possible, administering grafts into a more vascular bed is preferred. However, the most vexing clinical challenges that face clinicians are those that require larger volume subcutaneous administration (facial augmentation, breast reshaping, and liposuction contour irregularities). As such, we sought to study graft survival capabilities in this more difficult and challenging location; for if research can better characterize how fat grafts behave/survive in a subcutaneous layer, we will be in a better position to affect positive change for bigger clinical challenges in the future.

Second, readers accustomed to reading clinical studies might criticize these conclusions based on the number of subjects employed. Certainly, any scientific conclusion is afforded more weight with a larger sample size. However, in this study the number of mice was chosen to generate the appropriate statistical power of 0.80 and was validated by the statistical significance demonstrated ($P < .05$). If, hypothetically, no statistical significance had been shown, it could be argued that in truth of fact, there was a difference between treatment groups but that this difference was not shown because of the small sample size. This was not the case with this study, because $P$ values reflected statistical significance (and did so with very little variance within groups); increasing the sample size would likely have simply made small $P$ values even smaller. More subjects would lend even more weight to the conclusions, but because statistical significance was reached ($P < .05$), the conclusions are still statistically valid.

**CONCLUSION**

In conclusion, this study showed for the first time that a resorbable matrix itself can play a critical role in the success of purified adult fat cells suspended in it, highlighting the importance of the stroma/ground substance surrounding grafted cells and the interaction between the two in a way not previously characterized. It suggests that manipulating the material surrounding the grafted cells may improve their chances for success. In this study, as in many scientific explorations, more questions are garnered than answers. It will be up to future studies

![Figure 9. Percentage of graft volume occupied by fibrosis at 3 months.](image)

![Figure 10. Presence of residual matrix (green), paraffin tissue section at 3 months. Intact adipocytes highlighted in yellow, fibrosis in red. A, Purified fat graft; B, Purified fat/PuraMatrix grafts; C, Purified fat/GFR Matrigel grafts.](images)
into specific cell matrix interactions, the role of individual and combinatory growth factors, and translational research in larger animal (and then human) models to fully explain the ins and outs of this process so that clinical recommendations in humans can be made. But by ultimately understanding it and gaining the knowledge required to fundamentally offer grafted fat cells the environment they require, the reliability and consistency of fat grafts may be better optimized, opening the door for larger volume grafts and more aggressive applications.

**DISCLOSURES**

Funding for this project was entirely provided by the University of Wisconsin Division of Plastic and Reconstructive Surgery. The authors have no financial interest in and receive no compensation from manufacturers of products mentioned in this article.
REFERENCES


Accepted for publication September 12, 2007.

Reprint requests: Justin Plasecki, MD, 2010 Broadmoor Dr. E., Seattle, WA 98112.

Copyright © 2008 by The American Society for Aesthetic Plastic Surgery, Inc. 1090-820X/$34.00 doi:10.1016/j.asj.2008.02.005