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FIRST REACTIONS

Building Scaffolds To Rebuild Kidneys

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A bioinspired scaffold to treat renal disease by regenerating renal tissue.

Kidney disease is on the upswing worldwide with increased incidence and prevalence noted. The overall prevalence of chronic kidney disease (CKD) in the general population is estimated to be as high as 14%. In the US alone, we have almost 470 000 people on dialysis, and these individuals could greatly benefit from other options. Transplants are in short supply making tissue engineering/regeneration options look attractive. The kidneys do have a limited capacity for regeneration[1] but are also prone to fibrosis and scarring after injury. The ability to promote a regenerative, rather than fibrotic, response to injury in the kidney could open up an important therapeutic possibility in patients with CKD. The paper by Lih et al.[2] makes an important contribution to this topic by optimizing artificial scaffolds for kidney regeneration.

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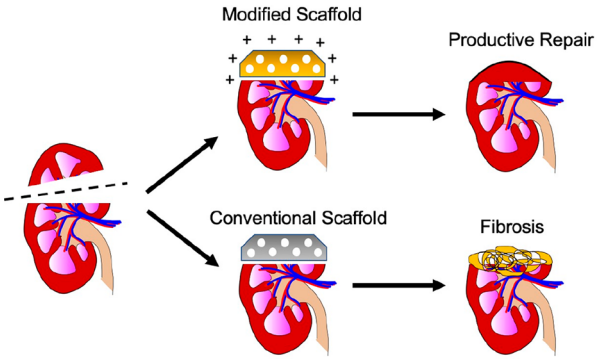
The rationale behind this study is the observation that artificial scaffolds of porous materials have been widely studied for their ability to enhance tissue regeneration, but commonly used polyester scaffolds such as poly(lactic-*co*-glycolic acid) (PLGA) release acidic byproducts as they degrade, which can have the opposite effect. The authors therefore seek to optimize these scaffolds, drawing inspiration from two examples of well-studied biology: the ability of magnesium hydroxide to moderate stomach acidity in antacid formulations, and the ability of extracellular matrix preparations to suppress immune responses and promote healing.[3] Note that magnesium hydroxide has already been used in tissue engineering scaffolds, for example.[4,5] Following these examples, modified scaffolds containing particles of magnesium hydroxide, porcine extracellular matrix (ECM), or a mixture of the two are combined with PLGA.



Modification of scaffold modulates kidney repair. Schematic depiction of the kidney injury experiment performed in Lih et al. Addition of magnesium (+ symbols) and ECM (yellow hue) to the porous scaffold leads to improved tissue recovery and reduced fibrosis after partial nephrectomy.

The resultant scaffolds have similar pore sizes (about 200 μm) as PLGA scaffolds alone, but exhibit increased hydrophilicity and thermal stability, properties that would encourage cell attachment and reduce degradation. More strikingly, inclusion of magnesium hydroxide into the scaffold noticeably stabilizes the pH of its surrounding PBS solution after weeks of gradual degradation. ECM alone has relatively little impact on pH, but in combination with magnesium hydroxide neutralizes acid degradation products with observed final pH from 2.0 (without $Mg(OH)_2$) to 6.5 with the salt present—a change of greater than 4 orders of magnitude in proton concentration.

Cell invasion of traditional PLGA scaffolds can be limited due to the relatively stringent biochemical requirements of cells for adhesion needed for migration and growth. To test whether their modified scaffolds provide a more optimal

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growth surface for kidney cells, the authors embed the scaffolds in a three-dimensional collagen hydrogel prepopulated with kidney tubular epithelial cells. After 72 h, the cells fail to invade the PLGA scaffold, but show significant binding to all three of the modified scaffolds, with maximal migration observed in the magnesium−ECM combinatorial scaffold. To summarize, characterization of the modified scaffolds to this point suggests two advantages: promotion of cell attachment in the short term, and neutralization of acidic degradation products in the long term. The combination of ECM and magnesium hydroxide appears to be optimal, based on these characterizations *in vitro*.

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In their final set of experiments, the authors compare the effects of the enhanced PLGA scaffolds on kidney repair outcomes after substantial nephrectomy (one kidney removed, and part of the other replaced with a scaffold). They find that incorporation of either magnesium hydroxide particles or pig kidney ECM into the PLGA greatly improves the recovery of the kidneys. Improvements noted include population of the graft with healthy tissue containing tubules and glomeruli, a reduced inflammatory cytokine profile, and serum biomarker readings indicative of improved kidney function during two months of follow-up. In certain assays, combination of magnesium hydroxide and pig kidney matrix results in a slight improvement beyond either one individually, suggesting that these may have synergistic beneficial properties, although for the most part all three of the modified scaffolds (magnesium, ECM, or magnesium + ECM) behave similarly. The authors thus conclude that modification of PLGA scaffolds with "bioinspired" features to reduce degradation and inflammation is a powerful strategy to promote a regenerative healing response within the kidneys.

Collectively, Lih et al. is an interesting paper with some potentially important conclusions. It builds upon previous work in the literature but goes to new territory. For example, it is known that decellularized kidney ECM can induce healing and regeneration.[6] It is known that fast degrading polyesters cause inflammation. It is known the $Mg(OH)_2$ can neutralize acid. Indeed, all the components of their development have been used before (PLGA, decellularized kidney, magnesium hydroxide, ice particle leaching). Nevertheless, it was a good idea to put all of these ideas together.

The study does raise certain questions, which will hopefully be addressed in future work. One issue that remains unresolved is how the scaffold becomes populated with healthy kidney tissue. The suggestion that new glomeruli—the blood filtering units of the kidney—form within the scaffold seems unlikely, as glomerulogenesis has already ceased by the time point at which the injury occurs (5 weeks), and it is well-established that kidney stem cells capable of generating new glomeruli cease to exist shortly after birth in mammals.[7] Rather, it seems highly likely that the scaffold promotes the recovery and survival of injured glomeruli, which perish in the presence of PLGA alone. Alternatively, the scaffold may actively inhibit the natural fibrosis response of the kidney, which would compete with the survival and growth of nephrons adjacent to the wound site.

The precise molecular mechanisms whereby the scaffold promotes kidney recovery are also not yet clear. It is suggested that the magnesium hydroxide serves to neutralize acidic byproducts of PLGA degradation, which are normally detrimental, while the ECM supplies growth factors to promote regeneration rather than scarring. These hypotheses could be tested by modifying these materials even further, in a controlled fashion, to determine the minimal characteristics that are necessary and sufficient for improved renal recovery.

The rapid release of acid noted here was in part due to the rapid degradation of the 50:50 glycolic acid:lactic acid PLGA used, a material that degrades so quickly that it is often not optimal for tissue engineering. Since the implication is that unmodified PLGA causes acidification of the lesion and is therefore detrimental to the recovery, it would be interesting to know what the effect is of leaving out the scaffold entirely. Further, wound healing environments are frequently acidic, and alkaline wound environments have been shown to delay or inhibit wound healing. How does the level of acid produced (or neutralized) play into this observation?

More broadly, the paper raises a number of interesting possibilities and future directions to explore. Our group has shown that pore size has a substantial impact on vascularization and cell population of scaffolds in different organs.[8,9] What is the effect of pore size here, and might it promote regeneration via a similar pathway? Could scaffolds such as these be preseeded with regenerative cell populations, such as iPS cell-derived nephron progenitor

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cells, which have the potential to differentiate into new nephrons *in situ*?[10]

Perhaps the most practical and pressing question is whether scaffolds like these would show similar efficacy in a clinical setting of partial nephrectomy, to preserve kidney function and reduce the organ's need for compensatory hypertrophy. Might the presence of magnesium hydroxide, or porcine ECM, produce adverse effects, which could outweigh the benefit of the modified scaffold? If so, are there other factors that could achieve the same effect, with fewer risks? There is much left to be determined on the road to clinical application. When it comes to "smart" biomaterials that guide healing and regeneration, one senses that we have only scratched the surface of what is possible.

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