Transforming surgery through biomaterial template technology

Templates inserted into surgical wounds strongly influence the healing responses in humans. The science of these templates, in the form of extracellular matrix biomaterials, is rapidly evolving and improving as the natural interactions with the body become better understood.

The extracellular matrix is a complex mixture of structural and functional proteins arranged in a tissue-specific, three-dimensional ultrastructure. Depending upon the tissue source, the extracellular matrix contains a precise composition of collagens, elastic fibres, proteoglycans, glycosaminoglycans and glycoproteins. Each of these components serves specific structural and/or functional roles that give the tissue a unique mixture of mechanical strength and elasticity, provide attachment sites for cells, and serve as a reservoir for growth factors and cytokines that modulate diverse host processes (Theocharis et al, 2016). The extracellular matrix is a dynamic environment that interacts with host cells to direct the inflammatory response following tissue insult (Sorokin, 2010), and plays an active role in inducing and maintaining angiogenesis and vasculogenesis during tissue repair following ischaemia (Cheresh and Stupack, 2008). In addition to acting as a scaffold through which cells migrate, the extracellular matrix can direct its own repopulation with specific cell types and can regulate its own turnover and metabolism (Alberts et al, 2002).

The extracellular matrix is a dynamic system that responds to the local cellular environment in addition to acting as a signalling reservoir for cells. In this way, not only are the local tissue cells affected by their surrounding extracellular matrix, but the cells are also active in secreting matrix and remodelling their surroundings in response to cues supplied by neighbouring cells and matrix-bound factors. This concept, often referred to as 'dynamic reciprocity' between the extracellular matrix and the cells which reside in it (Bissell and Aggeler, 1987), has become a widely-accepted paradigm in biology that has recently been translated to the field of tissue engineering in an attempt to apply it in the design and manufacture of therapeutics to treat human disease (Mauney and Adam, 2015).

Weaknesses of currently-used approaches to replacing tissues lost as a result of disease, genetic deficiencies or trauma have motivated researchers to engineer replacement tissues and organs. As the field of tissue engineering has matured, the focus of attention has shifted dramatically from synthetic, inert materials to truly bio-interactive materials for reconstructing replacements. The extracellular matrix has often served as the template (Brown and Badylak, 2014).

Collagen scaffolds readily remodel into living tissue and direct the development of living, functional replacements for damaged or diseased tissues and organs without the added complexity and undesirable aspects of living cell implants (Chan and Leong, 2008). Such materials have been used since the 1960s when it became obvious that the extracellular matrix actively participates in tissue development and response to injury. More recently, acellular biomaterials have been developed to mimic the structure and composition of the natural tissue environment (Hiles and Hodde, 2006). These materials have been tailored to orchestrate a predetermined host tissue response that continuously interacts with and responds to the local tissue microenvironment. These biomaterials may comprise whole extracellular matrix structures with an intact, native three-dimensionality retained from the host, or they may contain matrix-mimetic peptides or proteins attached to defined, naturally-occurring extracellular matrix scaffold structures, such as fibronectin, fibrin or keratin (Belcarz et al, 2009; Wang et al, 2013).

Such materials have been designed to present adhesion signals to provide for migration and signalling between the matrix and invading cells, to possess protease-sensitive sites for material degradation during constructive matrix remodelling by cell-associated enzymatic activity, and/or to contain matrix-bound growth factors to initiate a favourable graft–host response (Boehler et al, 2011). These materials can be used to induce a productive remodelling response in a variety of tissue engineering applications if the patient response to these materials is well understood (Morais et al, 2010) (*Figure 1*).

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Patient response depends on the local tissue environment

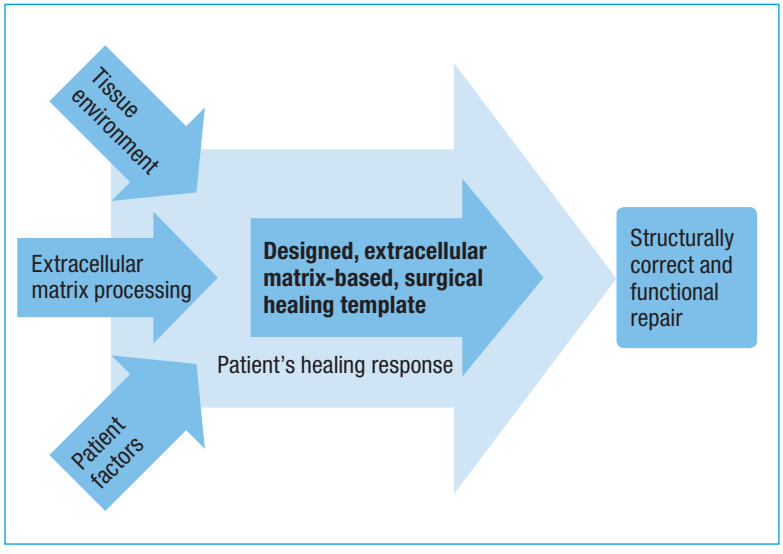
The human body has a remarkable ability to heal, but less well known is its equally remarkable ability to regenerate lost tissues – if the correct template is provided. In the mature adult of virtually all species, the wrong template (or no template at all) results in wound healing with scar formation and incomplete or at least incongruent tissue repair (Druecke et al, 2004; Liang et al, 2006). In order to coax the adult healing response down a more desirable, less fibrotic pathway, new surgical biomaterials have been created based on extracellular matrix (Liang et al, 2006). It is true that many other factors, such as surgical technique and mechanical stressors, affect the tissue responses, but these other factors are often secondary to the effects of the implant and its direct interaction with its surrounding environment following implantation (Scully et al, 2015).

The effects of the implant, especially if biologically active, are profound. While any extracellular matrix used as an implant acts as a guiding template for tissue remodelling as a result of the dynamic reciprocity provided to the patient's cells by its retained three-dimensional ultrastructure and microstructure, the precise signals and cues contained within it direct how the cells will react to it (Nihsen et al, 2008). Since 'like becomes like', it is apparent that a native tissue scaffold containing signals normally found in dermis will act as a template to form normal dermal-like structures once it has been implanted to reinforce a tissue defect. Similarly, an extracellular matrix scaffold derived from stronger, less elastic tissue – such as ligament or submucosa – will dictate a remodelling pathway that is more tendon or submucosa-like (Zhang et al, 2011). Differences in extracellular matrix processing can somewhat influence the ultimate outcome, as noted below, but implanting the wrong extracellular matrix into the wrong local tissue environment cannot be overcome through the effects of processing alone (Jiang et al, 2015).

Patient response depends on extracellular matrix processing

Many authors have contemplated the properties of the ideal biomaterial, but the 'correct template' may be something entirely different (Gigliobianco et al, 2015). With the goal of scarless, non-fibrotic healing and recapitulation of functional tissue, many of the biologically mute materials are much less than ideal. When cells of a wound encounter a synthetic, plastic material they do not see something familiar and hospitable. Instead, they react by setting up inflammatory processes that stimulate neutrophil and macrophage invasion, reactive oxygen species production, and overall tissue destruction (Bryan et al, 2012; Gigliobianco et al, 2015). As the inflammation subsides, fibrous tissue is formed to encapsulate the implant and separate it from the recipient as best as possible. Thus, pure synthetics act in a mechanical way to provide tissue connections, but their biological response is in direct conflict with the idea of a correct tissue template; the resulting tissue formed is mechanically stable fibrotic scar tissue rather than truly remodelled structural tissue that is mechanically and functionally identical to the structure it was meant to replace (Hiles and Levitsky, 2005).

Figure 1. The patient's healing response must integrate, around and through the extracellular matrix-based, surgical healing template, all of the factors from tissue environment, extracellular matrix processing, and patient-specific factors to achieve a successful outcome with a structurally correct and functional repair. It is a complex interaction that does not always work, in part because the state of the science is still lacking.



An alternative to fully synthetic scaffold materials is collagen-based, extracellular matrix-derived scaffolding. While many different extracellular matrix scaffolds are harvested directly from humans and other animals, processing methods and subsequent surface modifications can influence the eventual tissue response in the recipient. Unfortunately, many of these methods are deleterious to the optimal tissue regeneration response and result in disorganized tissue deposition and negative immune system response, causing an undesirable effect in the recipient (Chin et al, 2012; McDade et al, 2013; Bryan et al, 2015).

For example, chemically modified extracellular matrices have been used in surgery for several decades. Glutaraldehyde fixed porcine heart valves have shown longevity of mechanical function in certain patient populations and anatomical locations, but their biological response is essentially mute and similar to those of pure synthetics. They are plagued by calcification (a sign of absent tissue integration) and simple mechanical fatigue caused by an 'un-living' substrate of proteinacious origin that cannot turn over and be replaced with fresh tissue because of its extensive chemical modification. In fact, research has shown that the effects of chemical crosslinking can be devastating for the biological context of an extracellular matrix (Tam et al, 2015).

More recently, multiple groups have shown that careful processing methods designed to retain biological context in extracellular matrix materials can allow these more complete templates to better interface with the human body even if they are from cross-species (i.e. animal) origin. Porcine extracellular matrices, especially, have found their way into clinical medicine where they are used for hernia repair, pelvic floor reconstruction, chronic wounds, dura mater, plastic surgery, colorectal fistulae and many other soft tissue repairs (O'Donnell and Lau, 2006; Bejjani et al, 2007; Trabuco et al, 2007; Seymour et al, 2008; Hiles et al, 2009; Cintron et al, 2013; Holubec et al, 2015).

The decellularization and sterilization processes used for extracellular matrix biomaterials are key to their in vivo performance (Zhao et al, 2014). The specifics of these processes are generally proprietary in nature and are thus difficult to replicate by the scientific community. However, there is still room for improvement because currently-available extracellular matrix scaffold materials are still not ideal for all patients in all situations, in part because the processing has not been tuned to achieve the best response (Wong and Griffiths, 2014).

For example, the ever-present desire to decellularize and 'purify' extracellular matrix scaffolds can lead to inadvertent biological consequences. The stripping of important biological information, such as the removal of all carbohydrates through processing, can result in a dramatic loss in the ability of such materials to integrate and form new tissue. Further, harsh processing techniques that break down larger macromolecules present naturally in the extracellular matrix can result in unwanted tissue reactions. Similarly, residues of detergents or other processing agents used can stimulate degradative inflammation and aberrant healing processes, leading to overt rejection of the implant, prolonged inflammation and foreign body reaction that can result in chronic pain or inappropriate tissue integration that can lead to eventual mechanical instability (Cissell et al, 2014).

Sterilization is the process of rendering a material free of bacteria, viruses and fungi for the purposes of preventing their transmission to the patient recipient of the biomaterial. Traditional sterilization is achieved using steam, heat, radiation or strong chemicals, but as most of these processes are clearly incompatible with most tissues if their structure and composition is to remain intact. Consequently, much time and effort has gone into the study of various sterilization methods, their effects on extracellular matrix, and their optimization to help prevent unwanted biological degradation. Both ethylene oxide gas and rapid electron beam radiation have minimal effects on extracellular matrix structure and function (Hodde et al, 2007), yet the limitations of these methods in terms of penetrating complex structures or particularly dense extracellular matrices necessitates further improvements.

Fortunately, the problems related to decellularization and sterilization of extracellular matrix scaffolds are solvable. Safe and effective extracellular matrix biomaterials currently exist that synergize with the patient's own healing response to create functional tissue repairs; however, alternative methods are still needed so that variations in immune and tissue response from patient to patient can be predicted, mitigated against and overcome.

Patient response depends on the patient

Designing a tissue implant that can lead to a predictable outcome in all patients is challenging because of the inherent biological differences between patients (Nalebuff, 1990; Engeland et al, 2009). In addition to obvious genetic variability between patients, one of the most challenging aspects that needs to be addressed when designing an optimal implant for a patient is the inherent variability of response within a patient that may not readily be apparent on clinical examination.

For example, a patient's health status on any given day can influence how the implant may interact once implanted. A patient who is immunocompromised at surgery, perhaps because of a transient viral infection, may respond differently than he/she would on a day when he/she is healthy (Ebrecht et al, 2004). Similarly, a diabetic patient with variations in blood sugar level from day to day might respond differentially to surgery on a day when his/her blood sugar is normal as compared to when his/her blood sugar is excessively elevated (Berlanger-Acosta et al, 2013). Additionally, literature suggests that patients who are in an anabolic state at the time of surgery may fare better during recovery than if they are in a catabolic one (Jones et al, 2011) – yet health-care providers continue to place patients in a catabolic state before their operation on the day of surgery by withholding oral nutrition to appease the anaesthesiologist. This simple change in nutritional status during surgery may negatively affect patient recovery and response to the extracellular matrix implant because of a state of transient malnutrition.

Designing an implant that works for everyone is similarly challenging because patients are often misleading or omissive about their health. Patients who need technologically advanced implants are generally not healthy athletes without comorbidities; they are patients with multiple organ system infirmaries resulting from a history of environmental and behavioural factors beyond the practitioner's control. For example, because cells and extracellular matrix interact in a process of dynamic reciprocity, the signals on the extracellular matrix must be able to direct the cells to respond appropriately. In cells that are 'drunk' as a result of the effects of their local environment – sustained high blood sugar or functionality in the face of chronic alcoholism, for example – optimal implant design that can direct an 'army of confused cells' becomes a very difficult challenge to overcome, regardless of how optimal extracellular matrix procurement, decellularization and sterilization are (Jung et al, 2011; Berlanger-Acosta et al, 2013).

Because the field of transformative biomaterials is still in its infancy and because of the realities of how health care is regulated and delivered in most places, fine tuning

the implant to the patient is not yet possible. However, it is this aspect of biomaterials and advanced manufacturing upon which the success or failure of individualized surgical medicine is going to rely.

Conclusions

Acellular templates, whether they be synthetic or biologic in nature, strongly influence the patient's healing responses. For biomaterial implants derived from extracellular matrix, the patient's response depends upon from where the implant was isolated, how it was processed and sterilized, and into which environment it was implanted. While the science of transformative medicine has made great strides since the days of glutaraldehyde cross-linked scaffolds, the field is still somewhat rudimentary and major gains are still to be made.

Today's extracellular matrix implants have evolved from being largely inert biomaterials – their goal now is to be fully interactive with the recipient through the natural process of dynamic reciprocity. This has been done by optimizing decellularization and sterilization processes to leave the extracellular matrix mostly intact, structurally and compositionally, when used as an implant. While these devices are successful in many patients, regenerative medicine has yet to develop a tissue implant that is optimally processed and tailored for a specific patient with unique genetic, environmental and behavioural factors that will proscribe the implant's outcome. The science of these implants, in the form of extracellular matrix biomaterials, is rapidly evolving and improving as natural interactions with the body and its constituent cells, tissues, and organ systems, become better understood. **BJHM**

Conflict of interest: Mr J Hodde and Dr M Hiles are employees of Cook Biotech, Incorporated.

KEY POINTS

- The body can regrow amazing tissues if given the right tissue template.
- Without the proper molecular context, surgical implants are always recognized as foreign invaders. Purified extracellular matrix and pure synthetics both suffer from this.
- A patient's response to an implant depends on the nature of the implant, how the implant is manufactured, and how able the patient is to respond to it.
- The field of truly transformative biomaterials is still in its infancy.

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