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Review Article

Infected animal models for tissue engineering

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ABSTRACT

Infection is one of the most common complications associated with medical interventions and implants. As tissue engineering strategies to replace missing or damaged tissue advance, the focus on prevention and treatment of concomitant infection has also begun to emerge as an important area of research. Because the *in vivo* environment is a complex interaction between host tissue, implanted materials, and native immune system that cannot be replicated *in vitro*, animal models of infection are integral in evaluating the safety and efficacy of experimental treatments for infection. In this review, considerations for selecting an animal model, established models of infection, and areas that require further model development are discussed with regard to cutaneous, fascial, and orthopedic infections.

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1. Introduction

While tissue engineering holds great promise in the restoration of normal structure and function in a variety of disease states, infection continues to remain a significant challenge for the field. Tissue engineering strategies classically involve cells (either implanted or recruited), a scaffold (synthetic or natural), and chemical signals (such as growth factors). Infection prevents normal healing and inhibits the success of tissue-engineered constructs. The inflammatory environment caused by infection diminishes the natural capacity of tissue to heal and alters cellular phenotype [1]. Foreign objects, such as implanted scaffolds intended for cellular infiltration, may also act as a safe haven for bacteria and can result in pathogenic colonization [2,3]. In addition, the regenerative effects of delivered growth factors, such as bone morphogenetic protein 2 (BMP-2), have been shown to be mitigated in patients with infection [4]. As a common complication associated with wounds and tissue defects, addressing infection is an increasingly important aspect of tissue engineering strategies.

Due to the complex interactions between host and pathogen, *in vitro* systems cannot faithfully recapitulate conditions of infection and tissue healing. Therefore, there is a need for animal models which reflect tissue infection for evaluating tissue engineering strategies. There are many critical variables to consider when choosing a model of wound infection. In general when choosing any animal model for tissue engineering, one must consider the size and nature of the tissue defect, the physiological and anatomical differences in wound healing between the model and human disease, ethics of animal experimentation, as well as pragmatic aspects such as costs and housing requirements. However, in the case of infected animal models, one must also consider factors such as the species of pathogen (type of inoculum), amount of pathogen (concentration of inoculum), inoculation vehicle, and how the course of infection should be monitored and validated. It is important to note that no animal model can completely recapitulate the human condition, especially in a complex disease state such as infection. As different animal models can reflect different aspects of the same disease, often multiple models (including non-traditional models) are necessary to thoroughly explore a tissue engineering strategy before approval for clinical trials [5]. This review will cover considerations when choosing an infected animal model as well as discuss established models available in several defects (cutaneous, fascial, and orthopedic) as examples.

2. Considerations in model selection

When selecting or designing an infected animal model to evaluate a tissue engineering strategy, it is important to consider the host animal species and strain, the host animal defect, the pathogen species and strain, the inoculum concentration and vehicle, and many other factors specific to the disease state of interest. It is critical to

Abbreviations: ATCC, American Tissue Culture Collection; BMP-2, bone morphogenetic protein 2; CFU, colony-forming unit; qPCR, quantitative polymerase chain reaction.

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fully explore previous infected animal models available in the literature and often necessary to conduct pilot studies to ensure an infection has been created that is self-sustaining but does not overwhelm the host. Highlighted below are some specific considerations that are broadly applicable for tissue engineering purposes.

2.1. Host animal and defect

Overall, general trends in animal species selection for tissue defects hold true for infected defects. For the purposes of this review, smaller species include mice, rats, and rabbits. Larger species include pig, goat, sheep, dogs, and non-human primates. In addition to model species, strain can be important given physiologic differences such as immune response to infection [6].

In general, small animals are less expensive to purchase and house, require less complex surgical and anaesthetic equipment for tissue defect creation, and present less regulatory challenges for use in research. Infected mouse models have several advantages. Mouse studies are, in general, relatively inexpensive and as a result, can be powered to make strong statistical conclusions. In addition, mouse models are appealing due to the genetic tools available that are relevant to infection, such as strains that have immune deficits or metabolic imbalances [7,8]. In addition, genetically-modified mice can provide useful tools for understanding pathophysiology – for example, mice modified to have fluorescently-tagged neutrophils have been used to study cutaneous wound healing in real-time [9]. Because mice are small, *in vivo* imaging of bacteria via bioluminescence or fluorescence is somewhat less challenging since the depth of light penetration is shorter than in larger animals. Finally, there is currently more literature available on small animal models of tissue infection, which is an advantage when selecting an infected model for study.

Larger species tend to more accurately reflect oxygen diffusion limitations, musculoskeletal loads, and permit infected defects more similar in volume and geometry to clinically-relevant sized tissue defects. However, larger species are also more costly, have extra housing requirements, and raise additional ethical questions. In particular, the use of companion animals (such as dogs) and non-human primates raise complex ethical issues and should be avoided if possible.

Once a species and strain is chosen, the intended defect for infection must be designed. While the infected defect should recapitulate the intended human disease state as closely as possible, it may not be anatomically accurate, especially in smaller animals. For example, a subcutaneous pouch model has been established in rats to evaluate different hernia repair meshes for infection prevention [10]. While these meshes were not applied in their anatomically-intended defect (across a tear in the fascia), the model still allows for biomaterial evaluation in an *in vivo* soft tissue environment with a relatively simple surgery in an inexpensive model. Although these physiologic models can generate preliminary data for tissue engineering strategies, animal models which reflect the correct anatomic defects ultimately need to be utilized before translation [11]. Attention to detail in surgical protocol design is necessary. Seemingly minor decisions can alter infection in an animal model – for example, the choice of local anesthetic can impact the course of infection [12]. External drugs may need to be delivered to create metabolic imbalances [13] or immune suppression [14,15]. After deciding on the appropriate defect for the animal model, the inoculum (pathogen, dose, and vehicle of infection) can be chosen.

2.2. Inoculum

The species of microorganism chosen for an infected animal model is specific to the human disease being studied. In very broad strokes, the most common aerobic pathogens can be classified as gram positive or gram negative (based on cellular wall staining) [16]. In addition, the role of anaerobic species in wound infection is becoming increasingly recognized [17]. These fastidious species present challenges in their culture and inoculation due to sensitivity to atmospheric oxygen, but due to their role in chronic wounds and multi-pathogen infection, infected animal models are more frequently incorporating anaerobic species [18]. Out of these three general classes (gram positive, gram negative, and anaerobic), the specific organism is chosen based on prevalence in human disease. For example, bone infection (osteomyelitis) in humans is most often caused by *Staphylococcus aureus* [2]. Therefore, the majority of established animal models of infected bone defects involve *S. aureus* as the primary pathogen. For infected tissue defects in which there is no clear most prevalent pathogen, multiple groups with different organisms are often utilized, such as a representative gram positive, gram negative, and anaerobic microbe [15,19].

In addition to species, the pathogen strain chosen can have a significant impact on the animal model. While strains can be obtained locally from clinical isolates, it is recommended that they are purchased from a commercial source such as the American Tissue Culture Collection (ATCC) for standardization. Many commercially-offered strains have information available regarding the source of the pathogen. Different strains within the same species demonstrate different virulence factors, antibacterial resistance patterns, and rates of biofilm production [20]. Biofilm, the extracellular matrix produced by microbes, inhibits the local immune response [21] and increases antibiotic resistance [22]. As many foreign body infections are caused by biofilm-producing pathogens [23], tissue engineering strategies which incorporate cell scaffolds may be particularly vulnerable to biofilm-associated infection. As new therapies specific to biofilm-associated infections are being developed [3], animal models of infected tissue defects with emphasis on biofilm formation have been established and continue to be improved [24–27].

Regarding the inoculum, the concentration of pathogens and delivery vehicle must be considered in selecting or designing an animal model. If establishing a new model or a new pathogen within an existing model, often the optimal concentration must be evaluated in a pilot study where pathogen concentrations are increased logarithmically [28,29]. In most defects relevant to tissue engineering, a sublethal infection is desired. Therefore, a concentration must be high enough to generate a self-sustaining infection but low enough so that the infection is localized and does not result in systemic disease or sepsis. While inoculum is most frequently delivered by injection of media into the wound, delivering bacteria in a physical vehicle increases virulence. Vehicles in the literature include a pathogen-seeded collagen sponge [28], infected dextran beads [30], or a pre-formed biofilm [24,31]. While hematogenous bacterial seeding of implanted materials has been attempted, this approach was unsuccessful in a rat model [32].

While the majority of animal models of infected tissue defects challenge wounds with one pathogen species at a time, human chronic wounds such as diabetic ulcers most frequently feature polymicrobial communities [18,33]. In addition, multiple species have been demonstrated to show synergistic effects and alter bacterial phenotype in animal wound models [34]. Efforts continue to develop animal models of infection with polymicrobial populations that remain stable over time [31].

2.3. Infection evaluation

A critical consideration in designing or choosing an infected animal model to gauge a tissue engineering strategy is the method by which the infection and healing are evaluated. For the sake of discussion, these methods have been divided into three categories:

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