Bridging the gap: engineering bone for skeletal injuries

For years, mechanical fixators, grafts and cements have provided a surgical solution to bone injuries. However, the inherent restrictions of these treatments have prompted the search for newer options that can fully repair bone and restore its function. But, what are these approaches? What is the future of bone repair? Tissue engineering has stepped up efforts to tackle this difficult topic.

Bone fractures constitute a very common medical injury; there is a 1-in-50 yearly chance that you will be affected by one. They are so common, in fact, that there is a good chance you have already been directly or indirectly affected by it: a friend, a family member, or even yourself. For example, in the US, fractures constitute only around 16% of all musculoskeletal injuries and have a combined annual cost surpassing US $2 billion. With all these statistics, it is no wonder the complete and efficient repair of bone injuries has been one of the main challenges in the fields of medicine and tissue engineering (TE).

Bone is a complex tissue (Figure 1), consisting primarily of a spongy inner layer and a more compact outer layer. Bone cells sustain and remodel the mineralized matrix of bone. Interspersed through this structure, there is an intricate network of channels, nerves and blood vessels that contributes to the rich biological environment of bone. Bone marrow, contained in the porous spongy bone layer, is responsible for red blood cell formation. Thus, bone has a very important role – not only in maintaining the musculoskeletal integrity of the body, but also the delicate balance, or homeostasis, of vascular and nerve systems.

What are the current medical options for bone injury?

Currently, there are several clinical approaches to mending bone injuries. Grafts (transplanted tissues) encompass the most common of these procedures, with autografts (same patient), allografts (same species) and xenografts (different species) being used in an attempt to repair the injured site. However, availability and biocompatibility tend to be challenges for the use of grafts. Synthetic fillers and cements have been used to fill in the space left by critical injuries, but mechanical mismatch and lack of functionality limit the scope of their use. Finally, there are methods that allow the body to heal itself (Figure 2). These include the induced membrane, wherein the host body is stimulated to create a membranous tissue that can be used to envelop a graft and enhance healing; and distraction osteogenesis, in which the bone is mechanically adjusted to allow slow repair. However, these techniques are hindered by their invasiveness, length of recovery, variability of repair extent and failure rates. External and internal fixators

Figure 1: Schematic of bone tissue. Blood vessels and nerves are an integral part of the internal bone structure.
have also been used, but are unfortunately subject to the same drawbacks. The limitations of current treatments have driven a dedicated TE research effort to construct a significantly improved, customizable solution to bone injury.

The growing field of bone tissue engineering

When discussing TE, it is important to understand its history. Pioneering scientists worked extensively on the development of new materials that could be used to match the physical properties of body tissues. Their focus gradually shifted towards materials that could recreate, at least to some extent, the biological environment of the body. Today, the paradigm for TE centres on restoring the biological functionality of the target tissue, for which the material frame is only part of the solution. It needs to be complemented with chemical signalling to induce a response from the body, and the introduction of cell types that can promote the growth of tissue and the ideal microenvironment for other cells to infiltrate and assist in remodelling and repair.

Bone tissue engineering (BTE) has also gone through these stages. Historically, the initial focus of bone repair by TE methods involved the use of dense mineralized porous scaffolds, or devices that could replicate the hard structure of bone while providing a suitable environment for cells to grow and proliferate. In the last few years, however, BTE research has moved to enhance the physical frame and chemical make-up of these devices with a robust biological function to facilitate integration of the device into the body. The scaffold, cells and chemical signals are combined in a particular configuration and a targeted purpose for complete bone healing.

Taking repair to the next level: bone-nerve-vascular tissue integration

As mentioned previously, bone exhibits a hierarchical structure, from a dense exterior, to a porous inside layer, to a hollow interior. At the same time, vascular and nerve tissue inside the bone shows a tiered organization: large blood vessels and nerves branch into smaller vessels and nerves that penetrate the bone, and in turn these branch into a network that essentially covers the inner hollow structure of the bone. When a critical bone injury occurs, these structures are disrupted. Replacing only the mineral structure of bone would leave the restoration of biological functionality incomplete: the perfusion of blood, providing oxygen and nutrients into the affected site, and the nerve impulse coordination of tissue function are vital. For total repair, the continuity at both the bone, vascular and neural interfaces must be re-established. In fact, restoration of blood vessel flow and nerve connectivity is fundamentally at the centre of any approach to tissue repair. It is so important that it has been identified by major government and research agencies as one of the most critical challenges for

Figure 2: Surgical methods utilized for bone injury treatment. (A) The induced membrane technique involves placing an autograft inside a tissue layer formed around a temporary spacer. (B) Distraction osteogenesis uses mechanical fixation devices (in this case, an Ilizarov apparatus) to promote bone healing.
medical science in the new millennium. Even NASA, for example, recently included vascularization as one of its Centennial Challenges for the preparation of long-duration aerospace exploration, offering a prize to those teams that can successfully recreate a functional vascularized organ tissue.

One strategy that has been proposed as a solution to bone injury is the concept of the graded vascular scaffold (Figure 3). This device would have a rigid 3D printed porous frame. Since it would be printed, its configuration could be precisely controlled to mimic the structure of bone. A large synthetic vascular-nerve bundle surrogate would be incorporated into the rigid frame. This would allow the immediate restoration of blood flow into and through the scaffold as well as signalling incorporation between the engineered surrogate and surrounding tissues. Finally, the void fraction of the frame would be filled with a cell-containing degradable hydrogel. The perfusion of blood would provide the right conditions for cell growth. Connection of the nerve system would provide the right conditions for cell function and movement. Bone cells would induce the formation of a mineral matrix, while vascular and nerve cells would constitute the basis for newly formed capillary and neural beds, respectively. All would be involved in the remodelling of the degradable scaffold into new tissue with properties closely matching those of the original.

The key process that will determine the success of the engineered, innervated, vascularized surrogates will be integration between engineered components and host counterparts, which should occur concurrently at multiple levels. The first one is the assimilation of the host bone tissue with the newly formed tissue. The second one involves the incorporation of the blood vessel surrogate to the host blood vessel system. The third one would be integration of the microvascular network inside the scaffold to the blood vessel surrogate and surrounding capillary beds. The fourth one would be the conjunction between bone and surrounding musculoskeletal tissues, such as muscle, tendon or cartilage, which all contribute to mechanical loading and other signalling regarding healing and function. The last one, which is the least studied and understood, but could be vital for local, organ or full system function recovery, would be reconnection of nerves in engineered bone tissue and host musculoskeletal tissues. If any biological connections are missing, integration will not occur in entirety, and the treatment will potentially fail, be delayed or compromised. Without connection of mineralized tissue, the scaffold will not be stable; without connection to the vascular system, the scaffold will not be viable. In addition to the vasculature connection for engraftment and survival of the engineered surrogate, chemical and electrical signalling, through local environments or the nervous system, could direct and accelerate the formation of functional tissue. This strategy, in fact, is applicable to any other engineered tissue, not only bone: integration between

Figure 3: A vascularized, innervated bone scaffold has an environment favourable for the formation of a blood vessel-nerve network to sustain the viability of newly formed bone tissue. After implantation, complete scaffold incorporation is achieved through surgical connectivity, capillary and nerve integration, and bone ingrowth.
the host and an engineered, living surrogate holds great promise for successful repair, true regeneration and function restoration.

**Future perspectives**

Complete healing of bone injury is a historically relevant medical challenge, and research has evolved slowly to meet this challenge. Today, we have a clearer picture of the focal areas that need to be targeted. As illustrated through this article, fixing a bone injury comprises more than just finding a substitute for the tissue that was damaged. Future strategies for bone repair must include both a mechanical component (provided by a strong frame) and a functional component (with chemical signals and precursor cells). An ‘ideal’ configuration of frame, cells and signals is still to be determined, and constitutes the basis of modern TE research. Moving to a clinical setting will depend on a combination of approaches in a single or sequential implantable solution. While current clinical techniques have their limitations, they can provide the basis for the surgical connection of a tough perfusible device that can quickly restore function, minimize further medical intervention and enhance the efficiency of the repair. As solutions come up, we start to bridge the gap in earnest – figuratively and literally.

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**Further reading**

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