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Regenerative Medicine in Orthopaedics: Microsurgery Achievements for Translational Animal Model

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Abstract

Purpose: Despite many scientific advances, Regenerative Medicine is still in the preclinical stages in many areas. In this article, we intend to discuss the role of microsurgery in the bench-to-bedside transition of such primary findings. **Method:** By searching the papers related to the history of Regenerative Medicine (RM) and the news of Tissue Engineering (TE) in orthopedics in Pubmed, Scopus, and Google Scholar databases, we accessed a complete archive of various topics related to this field. **Result:** We first assessed the history and achievements of regenerative medicine, then we realized the importance of translational medical sciences and the role of animal models in this incipient phenomenon. Finally, after mastering the capabilities of microsurgery and the useful contribution of this technique to the advancement of clinical applications of regenerative medicine in various branches such as skin, skeletal system, nerves, and blood vessels, we decided to express the gist of our studies through this article. **Conclusion:** Considering the widespread use of small animals in regenerative medicine projects and the inevitable role of microsurgery in performing the best intervention on these animal models, the significant progress of regenerative medicine clinical application requires special attention to microsurgery in associated research.

Keywords

Regenerative Medicine, Tissue Engineering, Translational Animal Models, Microsurgery Achievement

1. Tissue Engineering and Regenerative Medicine

From the time of ancient Egypt, about 1500 BC, man has always dreamed of finding a way to repair or revive the damaged organs. One of the first attempts was blood transfusion [1] and after that, beginning in the 20th century, the fundamentals of organ transplantation in humans have developed rapidly. [2] Despite numerous progress in this field, the inherent problems of the organ transplant and limited donor resources have shifted many scientists to Tissue Engineering and Regenerative Medicine (TERM) as an alternative solution [3]. Tissue engineering is a science that has been developed to restore, maintain, and improve biological tissues, or a whole organ, using scaffolds, cells, and engineered materials [4] [5]. Regenerative medicine has a broader definition and is an interdisciplinary field of research that focuses on functional recovery or improvement of the damaged cells, tissues, and organs by repairing, replacing, or regenerating them [6] [7] [8] [9]. It needs a multidisciplinary team of physicians, engineers, and basic scientists with various expertise such as molecular biology, genetics, cell therapy, tissue engineering, immunology, and biochemistry [10] (**Figure 1**).

In recent years, various studies have been performed on the fabrication and implantation of bioengineered tissues in humans, including the skin [11] [12], cornea [13] [14], cartilage [15] [16] [17] [18], bladder and urinary tract [19] [20], respiratory tract and lung [21] [22] [23] [24], nervous system [25] [26] [27], heart and vascular system [28]-[35], liver [36] [37] [38] [39] [40], pancreas [41] [42] [43], etc. These studies provide an overview of the unique potential of regenerative medicine for the treatment of various diseases.

Despite various achievements, the knowledge of regenerative medicine is still in its infancy and many fundamental questions should be answered, including the selection of cell sources, development of tissue-specific materials, development of specialized bioreactors, and construction of complex organs [44]. In addition, much of the knowledge generated in this area has remained in the *in vitro*

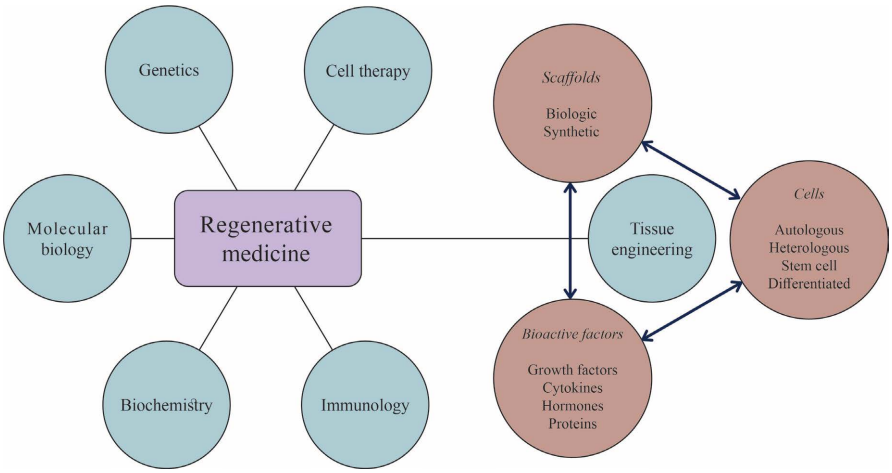

Figure 1. Tissue engineering and regenerative medicine (TERM).

stage and only a small percentage enters the *in vivo* stage, and subsequently, the clinical phase. This passage from laboratory to the human body is a long path that requires resolving numerous critical issues such as new processes formed by tissue-engineered materials in the body, differences between these processes with the natural tissues, material transformation, and the final destination of these products [44] [45]. This bench to bedside translation is what we are going to discuss here.

2. Translational Medical Sciences

It may take years, or even decades, before the findings of laboratory research can be applied in clinical practice. The average time for a medical intervention to successfully complete the clinical trials and enter the practice is estimated to be 17 to 24 years, while only a small portion of experimental knowledge passes the whole process [1] [2] [46]. The term "translational research" appeared in the early 1990s, which can be defined as the process of transforming the results of findings in laboratory, clinic, or community, into an intervention to improve public health [2] [47]. In 2009, the National Institutes of Health (NIH) introduced two areas for translational research: 1) developing human studies from the lab discoveries and preclinical findings, and 2) enhancing the implementation of best practice in the community [48]. According to this definition, also known as the NIH Roadmap, translational research is an ongoing process with the final goal of improving patient health.

The main components of the NIH roadmap are translational steps. The first phase of translational research (T1) is a bridge between the knowledge gained in the laboratories regarding the mechanism, diagnosis, prevention, and treatment of diseases and performing the first human assessments. In other words, the T1 phase fills the gap between the basic research and early phase clinical trials. The lab findings obtained by animal models, cell culture, and molecular studies should be prepared and standardized before use in clinical trials, which is typically referred to as "bench to bedside." The next phase (T2) transfers the results of clinical trials to daily clinical application and industrial use (commercialization) with the ultimate goal of improving the health of individuals and society (**Figure 2**) [2].

Preclinical and basic science researches are the first step in the NIH roadmap and the translational research process. They provide a foundation for clinical research using *in vitro* and *in vivo* environments [49]. Although in vitro methods provide a controlled experimental environment, they occur outside of a living organism and may fail to reflect the normal physiological condition. In regenerative medicine, such experiments commonly focus on cell culture and the application of cultured cells to investigate basic biological mechanisms, cellular activity, cell-cell interaction, and cell toxicity. On the other hand, *in vivo* experiments can simulate a physiological environment similar to the human body. Common applications of *in vivo* studies are the assessment of transferred cells and tissue

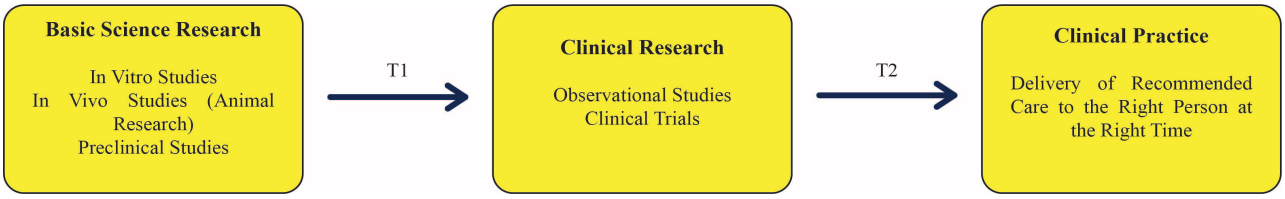

Figure 2. The current National Institutes of Health (NIH) Roadmap for Translational Medical Research.

survival, regional distribution cellular kinetics (e.g., proliferation, migration, differentiation, matrix synthesis, apoptosis, necrosis), implanted scaffolds biocompatibility and degeneration, cytokine response after an intervention, and drug safety and efficacy [3] [4] [5] [6]. Similar to other fields of health sciences, regenerative medicine needs to follow this translational process and requires reproducible models that can be used in both *in vitro* and *in vivo* studies [8].

3. Animal Models

Today, more than 20 million animals are used in health research, mostly mice and rats [50]. Although in vitro studies have been greatly enhanced due to the recent advances in technology, in vivo studies continue to play a key role in regenerative medicine. One of the greatest challenges of animal modeling is to choose the appropriate model that fits the goals and settings of the study. Animals are physiologically and biologically similar to humans, and also, are susceptible to many of the same health issues [51]. In the early years of the 20th century, domestic rats were the first animals to be used for research. Today, the main use of animal models is for translational medicine and these models are the mainstay of studies on the pathophysiology of diseases, diagnostic approaches, and therapeutic interventions.

No animal model can precisely simulate the complex conditions of the human body, but there has been no alternative to in vivo models so far. According to Isselbard *et al*. seven characteristics are listed for an ideal animal model [52]: 1) it must represent a valid human disease, 2) it should be available and easy to access, 3) its size should be sufficient for the sampling of biological specimens, 4) it should be easy to handle, 5) species and subspecies of that animal model must be available, 6) it must have a sufficient lifespan to conduct research, and 7) the animal model should be robust for the purpose of the study.

In 2010, Muschler *et al*. have presented basic criteria for an effective tissue regeneration model [53]: 1) It should provide an environment that is as compatible as possible with the clinical and biological environment and the formulation of the materials in which the evaluated methods are used, 2) it should provide objective and measurable parameters to evaluate the success (quantity and quality) and performance of the regenerated tissue, 3) it should find and predict clinical differences in physiological performance between methods.

Many variables associated with *in-vivo* animal studies can reduce the sensitivity of the study which should be controlled to reduce the number of animals for

an ideal statistical power. These variables are categorized into different fields such as animal-related variables, environmental and nutritional conditions, anesthesia, type of interventions or drugs, follow-up conditions, and type of assessments.

In 1959, Russell and Burch introduced principles of human experimental techniques and the 3Rs (Replacement, Reduction, Refinement) [54]. According to this concept, if it is possible to use smaller animals for the desired model, larger animals should not be used. The choice of animal models depends on the research question and stage of the research. Small animal models are commonly used in the discovery phase, while large animal models are needed for preclinical studies [55]. Due to the possibility of using more animals and confirming the study by repeating it, small animals allow us to obtain more reliable results. Small animals have a relatively shorter life cycle and can be studied throughout life or even for several generations. In addition, the environmental conditions of the animals are easily controllable, which is more difficult with large animals. Perhaps the most important problem with small animals such as rodents is the small anatomy that especially affects the field of surgery by restricting the use of routine tools.

4. Microsurgery

Microscopic surgery has long been used in complex procedures, including organ reattachment and human transplantation. The tools used in microsurgery allow surgeons to have very small structures in their area of intervention, which are not normally visible to the human eye [14]. In recent years, the importance of microsurgery in biomedical research has increased exponentially due to the advent of extraordinary micro-instruments and the undeniable efficiency of micro-sutures in successful anastomosis of small vessels and nerves sutures [56]. The use of microsurgery allows researchers to use small animals like mice as a surgical model in regenerative medicine. Researchers have benefited greatly from microscopic surgical techniques to make the technically impossible translational models happen. Researchers divide the science of regenerative medicine into two branches in terms of application, with scaffold and without scaffold. The first category is called cell therapy, which is more related to internal medicine, and the second category is tissue engineering, which is obviously related to surgery. Therefore, considering the many capabilities that microsurgery has in preparing basic requirements, processing the products in living cases and even performing major surgeries more perfectly in the transplantation of small vessels and nerves of engineered organs or tissues, it does not seem excessive to use the phrase of "regenerative microsurgery supported by tissue engineering" for this category [15]. Performing surgical procedures on small animals can reduce the need for traditional surgical models of bigger animals such as dogs and pigs, which are generally associated with more ethical and technical challenges [16].

In the following, we present the application of microsurgery in regenerative

medicine for skeletal systems and orthopedic related issues by mentioning some examples.

4.1. Skin

Skin was one of the first areas noticed by regenerative medicine, so various animal studies were conducted to find natural and artificial grafts for skin defects, burns and diseases. Researchers have tried to use different types of stem cells and cell lines to produce a complete skin tissue with all the skin appendages, such as sweat gland, hair follicle, sebaceous gland [57]. Among these, we can mention the study of Zhenggen Huang on mice, who achieved a useful model for healing burns and wounds by creating a defect in the skin of mice and using Porcine Embryonic Skin Precursors (PESP) in the lesion by microsurgery [58].

One of the important concerns in skin regeneration is the provision of a suitable scaffold for the placement of cells and their growth and transfer [59]. Keith A. Blackwood presented 2 models of PLGA 85:15 and 75:25 as the preferred models among 6 types of experimental electrospun polymer scaffolds on rats after comparing the grafting results [60]. Pierre-Luc Tremblay designed a human endothelialized reconstructed skin using the knowledge of *in vitro* prevascularization, and by transplanting it in rats, they found it useful for accelerating vascularization in skin grafts [61]. It is clear that there are many such small animal studies, and we will limit ourselves to mentioning only a few examples. The importance of microsurgery in skin regeneration is that these scaffold grafts are very delicate and unable to withstand the high tension of the suture, so with microsurgery, it is possible to have a more successful graft in small animals and even humans [62].

4.2. Bone

In orthopedics, microsurgery has been commonly used for the critical bone defect model (a defect that will not improve without intervention) [63]. This model has been described in both large (e.g., dogs, sheep, pigs and goats) and small animals (e.g., rats and rabbits). The main advantage of large animal models is the bigger dimensions which increase the accuracy of modeling and decrease the complexity of surgeries. However, these models have a longer lifespan and require more study resources. New microsurgery techniques and tools have made it possible to use small animals with an accuracy comparable to larger animals for bone defect studies [64] [65]. Additionally, Mice are favorite animals to study bone and its regeneration, because not only do they have similar mechanisms to humans, but in studies related to osteoporosis, researchers can easily ovariectomized them by performing a simple microsurgery and involve them into various projects [66]. For example, Livia Poser used Bone Tissue Engineered Constructs in the femur of normal and osteoporotic rats to investigate the effectiveness of the product and compare the regeneration process in the two groups. Providing a suitable environment for the growth and performance of regenerative medi-

cine products is a very important point that has become more available thanks to microsurgery [64]. Wei Fan achieved a new model of tissue engineered periosteum by inserting a special type of bone marrow stromal cells in mice that has been cobalt chloride-treated, which was useful for improving malunion [66]. Although the effect of platelet rich plasma on bone healing in human studies is still controversial, combined treatments have significant benefits in this field. Among them is the study of A. Meimandi Parizi, who provided a useful combination for major diaphyseal defects by injecting human platelet rich plasma plus Persian Gulf coral in rats that underwent radius osteotomy [67]. As it is known, microsurgery is necessary both for the production of the regenerative product and for creating defects in the studied animal.

4.3. Cartilage and Growth Plate

Approximately one-third of fractures in children are associated with growth plate involvement, which may lead to defective longitudinal bone growth. As a result of the damage to the growth plate, it undergoes premature ossification and production of a bony bar at the site of injury [68]. This event causes stoppage on one side of the growth plate and leads to limb deformity. The use of microsurgery has made it possible to create a model of growth plate injury in rats, which would not have been feasible without this technology, due to the extremely small size of the growth plate in rats. As Christopher B. Erickson discussed different treatment strategies for this lesion with microsurgical induced injury to the tibial growth plate in rats. It should be noted that even increasing knowledge in the field of regenerative medicine, which is a step before its clinical application, is also facilitated by microsurgery [69]. Rosa Chung by injuring the growth plate in mice intentionally via microsurgery and subsequently examining the cases in terms of gene expression, histological and immunohistochemical analysis achieved to important information in this field, which can be used in tissue engineering for Growth plate repair in children [70] [71] or Fiona H Zhou who through the same procedure, found the usefulness of TNF-α in the repair of growth plate injury [72]. In practical studies, Sang-Uk Lee harvested cartilage cells *in vitro* and used it in iatrogenic growth plate defect in rabbits whose results indicating the usefulness of this method to minimize deformity during bone bridge formation [73].

4.4. Nerve

Nerve reconstruction is a topic of interest to researchers in regenerative medicine, hoping to treat irreversible damage to the central nervous system and peripheral nerve damage. The modeling of peripheral nerve damage in small animals is another example of the impact of microsurgery on translational research [74]. Aleksandra M. McGrath compared Nerve Transfer in Proximal and Distal modes with repeated nerve transplantation in rats, which led to valuable information in this field [75]. The use of microsurgery techniques is not limited to the

creation of small animal models. It has enabled the scientists to produce tissue engineering and reconstructive medicine products such as scaffolds and cells in much smaller volumes and dimensions, and furthermore, to assess them with higher accuracy. For instance, peripheral nerve repair models such as median nerve injury in rats have been dramatically improved with microsurgical techniques. Now we are able to assess different types of bioartificial tubes on the median nerve injury models in rats [76]. Martin Lietza, who studied on neuro-tissue engineering from different aspects and evaluated types of nerve guides, contributing factors in nerve regeneration and cells used for repair in animal studies on mice [77]. Another researcher, Nektarios Sinis *et al*. has investigated the possibility of Cross-chest median nerve transfer, the use of a resorbable nerve conduit for nerve repair with the help of Schwann cells and other methods on rats in separate articles [78]. All of which included microsurgery as the main key for nerve transplantation, creating a deliberate defect in the nerves and test various tubes to guide the nerves growth [79]. There are many other studies in this field, although there is not any gold standard method, these studies on small animals have significantly increased knowledge and it is hoped that with the help of microsurgery, greater achievements will be made in this field and Central Nervous System (CNS) regeneration in the near future [80].

4.5. Vasculature

Vessels have been one of the most successful fields in tissue engineering, which has provided many natural and artificial models for large, medium and small vessels, and scientific progress in this field continues. Most clinical trials in this field have already been tested in animal studies [81]. For example, Marc Chaouat showed the efficiency of a polysaccharide-based scaffold as a small-diameter arterial replacement by microsurgery grafting in the aorta of rats [82]. In more advanced models, in a study on rats, Wei Wu transplanted a new type of fast degrading elastomer in abdominal aorta, which remodels from a cell-free synthetic graft into a neo-artery [83].

One of the main limitations in the manufacturing of tissue engineering products is the creation of a suitable vascular bed. Since providing adequate blood supply in the early stages of life is crucial for bioactive structures, four main methods have been designed to for perfect vascularization including scaffold design, *in vitro* prevascularization, in vivo prevascularization and angiogenic factor delivery [84]. To address these shortcomings, alternatives such as in vivo reactors have been introduced to use the body's potential to produce vascularized tissues of the desired size and shape in an implanted chamber. To improve the geometric accuracy of the final structure, a non-biodegradable custom chamber is filled with osteoconductive or osteoinductive materials and is surgically implanted in the recipient's body away from the defect site. A vascularized autogenous bone is produced within a few weeks according to the chamber's geometry, which can be harvested with the vascular stem and transferred to the bone

Table 1. Microsurgery achievements for translation of regenerative medicine.

1	Studying small animal more conveniently	Replacement, Reduction, Refinement
		Similar physiology and biology to humans
		Easy to handle and manage its environment
		High sample number and reproducibility of the study
		Shorter life span and follow up time
2	Production of regenerative medicine products	Preparation of graft and Scaffold
		Production of growth factors by xenograft preparation
		Inducing defects for study
		Exact sampling
3	More precise replacement	Scaffold attachment
		Proper vascularization technique
		Efficient nerve and vascular transplantation during major surgeries

defect. For example, A.S. Bigham by insertion of demineralized bovine fetal growth plate powder in the stomach of rats, recognized the osteoinductivity of this substance and its usefulness as a xenogenic graft in bone healing defect [85]. In other studies, Sauli Kujala inserted a weight-bearing nickel-titanium bone graft substitute as a scaffold in rats and compared the different components of this implant for the osteointegration [86]. Although F. Kurtis Kasper achieved the same Tissue Engineered Prevascularized Bone model with sheep surgery and Soft Tissue Flaps insertion [87], but with microsurgery, such interventions can be performed on small animal models with less cost and time. **Table 1** summarizes the achievements through which microsurgery has promoted the translation of regenerative medicine.

Despite the limitations associated with microsurgeries such as expensive equipment, unavailable infrastructure, and lack of skilled surgeons in this field, our findings show that tissue engineering has led to myriads of significant achievements in medicine by applying microsurgery so far. This fact confirms that to have conducted successful creative projects in regenerative medicine, a skillful microsurgeon in the research group must be involved and proper facilities in multidisciplinary research should be provided for this major.

5. Conclusion

Tissue engineering and regenerative medicine have great potential for the fundamental improvement of translational medicine and disease care standards. To overcome the obstacles in this journey, biomedical research has greatly benefited from microsurgery. The use of small animals, due to the relatively shorter life cycle and easier housing conditions, as well as the small nature of structures that are the target of interventions in animal models have resulted in increased ap-

plication of microsurgery in recent years. Therefore, to achieve more success in the field of regenerative medicine, the presence of a skillful microsurgery group in all stages of research, trial and human study is necessary.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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