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A medical device for prefabrication of large bone grafts in modern medicine

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ABSTRACT

Translating advances in the laboratory into sound clinical practice presents a series of formidable conceptual and technical challenges. One of them is our inability to maintain large grafts of living cells upon transfer from *in vitro* conditions into the host *in vivo*. This is due mainly to diffusion limitations within the grafting material. We embrace the well-known hypothesis of the "Diamond Concept" in bone tissue regeneration, which includes four key factors. Based on the understanding of basic elements of tissue engineering constructs, prefabrication and conditioning techniques and the nano-vascularisation of the scaffold, we furthermore hypothesize that combinations of cells, solid multipolymeric scaffold as the "core element" working as the extracellular matrix (ECM), growth factors and nano-vascularisation setting may eventually generate a large "ready-to-use" *in vitro/in vivo* graft. We are confident and think that growth factors will help in the construction of a step-by-step organisation of the bone tissue engineering construct (BTEC). A medical device, named *in vitro/in vivo* Bone Bioreactor Tissue Engineering Construct (IV2B2TEC), is proposed to fulfil the hypothesis. Soon, we hope to test the above hypothesis on a non-union bone defect in an animal model. This novel strategy will likely open new options for reconstructing extended bone defects and facilitate clinical translation of bone tissue engineering. As compared with conventional reconstructive methods, the strategy has four key advantages and might prove to be a novel armamentarium for clinicians in regenerative medicine.

Introduction

"*All truth passes through three stages. First, it is ridiculed. Second, it is violently opposed. Third, it is accepted as being self-evident.*" (Arthur Schopenhauer).

Restoring the normal physiology of a part of the body damaged by physical, chemical or ischaemic insult, or as a consequence of infections or genetic disease, is the essential aim of regenerative medicine. Many challenges confront tissue engineering, a rapidly developing field that has encouraged very productive interdisciplinary collaborations between the physicochemical and biological sciences. Translating advances in the laboratory into sound clinical practice presents series of formidable conceptual and technical challenges [1]. A critical issue in tissue engineering is the inability to maintain large grafts of living cells upon transfer from *in vitro* to *in vivo* conditions. Most cells do not survive more than few hundred micrometers from the nearest capillary, due mainly to diffusion limitations [2].

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IV2B2TEC: an integrated strategy for prefabrication of large bone grafts

Engineering bone constructs *in vitro* with a pre-existing vascular component is a major challenge. It faces one major limitation related to the survival of cells in the large constructs is minimal after they are transferred to an *in vivo* environment [3]. This challenge hampers, up to now, initiatives to engineer large and complex tissue. Nevertheless, several endeavours were dedicated to engineer bone *in vitro* by using stem cells and growth factors in bioreactors [3]. Unfortunately, the lack of oxygen and nutrients at the early stages is impacting viability of the implanted cells and limiting bone formation in large bone defects. Bioengineers have used an *in vivo* bioreactor for fabrication of a large mandible replacement in a clinical practice [4]. A prepared transplant composed of xenogenic bone mineral blocks infiltrated with recombinant human bone morphogenetic protein (BMP) and autologous bone marrow which was implanted into the patient's *latissimus dorsi* muscle to generate a heterotopic bone graft, after a 7-week prefabrication period [3]. Numerous studies indicated a novel engineering approach to create previously non-existing bone tissue ectopically.

"*To raise new questions, new possibilities, to regard old problems from a new angle require creative imagination and marks real advances in science.*" (Albert Einstein).

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Building a scaffold: the fishing unwinding approach – let's put the cells into it!

If we believe that "The art of scaffolding is where to put the holes" [5], thus the art of tissue bioengineering is: ***How to place cells within the heart of the scaffold***? We chose the electrospinning method, one of the three different methods employed in the fabrication of nanofibrous scaffolds for tissue engineering. Electrospinning is a century old technique that has proven to be simple, the most cost-effective and a highly adaptable fabrication strategy that can be scaled over a wide range of production values, factors critical to commercial and clinical success. Various fibrous assemblies can be constructed using electrospinning [6]. It is, however, difficult to create 3D scaffolds with a well-defined architecture and complex geometries, including porous interconnected networks. Initial attempts at combining electrospinning with 3D printing have yielded some success, but further assessment of this process is needed [7].

The design of the scaffold prior to exposure to cells is of vital importance. The scaffold must present a structure that promotes cell attachment, growth and differentiation, while providing a porous network for tissue ingrowths. The material chosen is of key importance when designing a scaffold. It must degrade at a rate matching that of new tissue formation (bone), it must be biocompatible and the products of its degradation must also be compatible. Once implanted, the scaffold must have biomechanical properties necessary to temporarily offer structural support until the new tissue has formed. To achieve these requirements, tissue engineering scaffolds are often designed to mimic the structure of the naturally occurring extracellular matrix (ECM). The fibrous collagen may play an important role in regulating cell attachment, proliferation and differentiation, and trends in scaffold design have aimed to better mimic this structure. In fact, with a combination of *in vitro* and *in vivo* cultures, nanofibrous scaffolds have produced bone tissue containing vascularisation, mineralisation and embedded osteocyte-like cells [7]. Therefore, we think that integrating stem cells and vascular elements along a highly original blend of silk/collagen/hydroxyapatite knitted fibered scaffold in an (automated) unwinding fashion is an innovative process of constructing an "organised" and "layer-by-layer 3D construct" suitable for bone tissue engineering. Correspondingly, Ayres and colleagues said that "the next generation of scaffolds may well function as templates that can be used to control cellular phenotype yet contain the information necessary to program the local cell population to remodel the microenvironment into the appropriate composition" [8].

Stem cells and growth factors

A stem cell is one that, through asymmetric mitotic cell division, is able to differentiate into several specialised lineages (multipotency), while retaining the potential for self-renewal [5]. The progression of cells from immature phenotypes to the highly specialised phenotypes present in tissues is a complex process governed by many factors. At present, combination therapies of stem cells and growth factor-release scaffolds tailored to promote angiogenesis and osteogenesis are under evaluation and development for the active stimulation of bone regeneration. Nanofibers have enhanced the differentiation and function of several cell types including fibroblasts, endothelial cells and osteoblasts. Cells, which are typically in the range of 10–100 μm in diameter, respond to stimuli from the macro environment down to the molecular level. Interaction between a typical cell and its environment is achieved through an array of receptor systems that are found on its outer membrane [6]. Also, as immature cells differentiate toward the osteoblastic lineage, they begin to express a sequential series of markers which culminate in the secretion of proteins like osteocalcin and bone sialoprotein and the mineralisation of the ECM. Alkaline phosphatase, an early marker of osteogenic differentiation, has been shown to be up regulated *in vitro* in cells cultured on nanofibrous materials compared to controls. Late osteogenic markers, such as osteocalcin and bone sialoprotein, have been shown to be up regulated in cells cultured on nanofibers [7]. Interestingly, engineers produced the desired thickness of cell-fiber multilayered structures completely within the electrospinning system [9].

Automated "biocreators": keynotes on kinetics/timing/controlling of the biological parameters with micropumps

Communication between the cell and ECM molecules influences various cellular processes, such as adhesion, proliferation, differentiation, migration, as well as growth factor and cytokine modulators [8]. Coordination among cell and molecular biology, molecular genetics, materials science, robotics and mechanical and electrical environmental engineering is mandatory, thus rendering the field multidisciplinary [5]. The timing of these events critically affects tissue formation and remodelling, processes that are crucial for the integration of a tissue engineering scaffold into the surrounding environment. The fibres that make up the backbone of the elaborate ECM network exhibit distinctive and, often times, regional variations in identity, cross-sectional diameter and polarity. In order to produce a functional scaffold, the complex and multifunctional nature of the ECM must be characterised and replicated. Due to the very short half life of growth factor (60–240 min), the direct and continuous application of the factors at the needed place is necessary for this process [10]. We are optimising the local drug delivery procedure, dosage and timing of the release of GF on differentiated human pulp stem cells in an *in vitro* model. To optimise the therapeutical outcome, however, the required dosage of growth factors and application systems needs further research. Electroosmotic pumps (EOPs) and their applications in microfluidic systems might help [11]. Electroosmosis requires charged solid surfaces to generate electroosmotic flow. In fact, a HA surface in contact with an aqueous solution becomes charged due to the deprotonation of the HA surface. Our model fits with the theoretical aspect of EOPs: EOPs are capable of producing flows at high pump rates and against high backpressures. These pumps are capable of generating constant and pulse-free flow in the bioreactor. The lab-on-chip devices have already been used in drug delivery for more than 10 years [12]. Properly designed, EOPs can be used as a stand-alone pump or integrated into a micro-chip device to generate flow rates and pressures for miniaturised development. Complex, fluid-like blood might be a problem in transport performance; it is therefore not known "*a priori*" whether the presence of complex fluid will lead to degradation or an improvement of the pumping performance. It remains a question to be addressed in the future [13].

Angiogenesis and nano-vascularisation

Nano-structuralised vascular scaffolds can be fabricated by phase separation, electrospinning and self-assembly of peptides of structural proteins, such as collagen and elastin [14]. The most exciting breakthrough in electrospinning is the successful one-step rapid fabrication of a vascular scaffold with integrated living cells. Despite this impressive progress in vascular tissue engineering with the help of innovative electrospinning technologies, rapid cell integration into scaffolds and determining their optimal mechanical properties remain the main challenges [14]. Vascular tissue engineering is one of the Holy Grails of tissue engineering. Still, the development of other novel, bioreactor-free methods of bone tissue growth, including vascular tissue assembly and rapid

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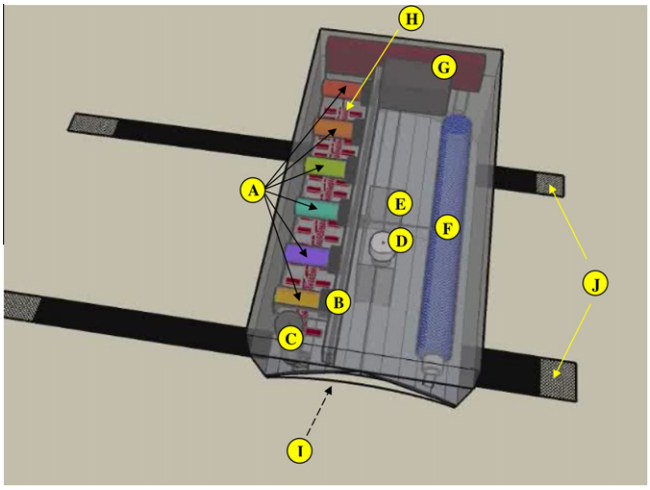

Fig. 1. The prototype of the IV2B2TEC. (A) Growth factors in recipients. (B) Micropumps (black squares). (C) Endothelial cells-seeding site (dark grey). (D) Stem cells-seeding site (light grey). (E) Stem cells chamber. (F) Electrospinning fibers (blue). (G) Electronics and motors. (H) Hydroxyapatite scaffold (red). (I) Intravenous connectors (under). (J) "Velcro" forearm straps. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper.)

vascular biofabrication, represent probably the most important breakthroughs so far, and they strongly indicate the potential of nanotechnology in tissue engineering for the development of commercially successful, cost-effective complete bone-vascular-tissue engineered products.

Presentation of the hypothesis

The "Diamond Concept" in bone tissue regeneration includes four key factors. Based on the understanding of basic elements of tissue engineering construction, prefabrication and conditioning techniques and the nano-vascularisation of the scaffold, we hypothesise that a combination of cells, a solid multipolymeric scaffold as the "core element", that works like the ECM, and growth factors and a nano-vascularisation setting may eventually generate a large, ready-to-use *in vitro* and *in vivo* graft within a short period of time (16–20 weeks). Growth factors will provide step-by-step organisation of the bone tissue engineered construct (BTEC). The medical device, IV2B2TEC (Fig. 1), will be automated and able to deliver GF via appropriate pumps under continuous flow and in physiological medium (under controlled pH, O_2, nutrients uptake and body temperature). Eventually, whole blood will precondition the construct with the aid of an arterio-veinous microcirculation from the forearm of the patient a few days before tissue transplantation. The protocol to test the above hypothesis includes implantation of the 3D custom scaffold that carries osteogenic cells differentiated from stem cells and vascular capillaries from endothelial cells into a non-union limb defect model in animal. This novel strategy will open new possibilities for reconstructing extended bone defects and facilitate clinical translation in bone tissue engineering if the hypothesis proves to be practical. As compared with conventional reconstructive methods, the strategy has the four following advantages. First, the volume and shape of TEC would be customised, and the osteogenic cells would be integrated inside the scaffold within less than 150–200 μm from newly formed capillaries by endothelial cells, as suggested in the literature. Secondly, grafting TEC to the bone defect would become a typical technique in surgery. Thirdly, it would promote bone healing in sites with poor blood circulation, particularly in post-radiated bone. Fourthly, it would be a novel armamentarium for regenerative medicine.

Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.mehy.2010.11.031.

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