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Controlling cell biomechanics in orthopaedic tissue engineering and repair

Contrôle de la biomécanique cellulaire au cours de la réparation des tissus ostéo-articulaires par ingénierie tissulaire

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Abstract

Tissue engineering offers an alternative approach with great potential for the treatment or replacement of damaged tissues or organs. In contrast to current treatments, a small sample of cells can be collected from the patient and cultured in vitro, greatly increasing the number of cells available for engineering tissue implants. As a result, engineered tissue implants limit the problems associated with patient trauma and undesirable immune response currently observed in surgical treatments practised in tissue and organ replacement. Mechano-transduction is known to play an essential role in bone tissue remodelling and repair. At physiological magnitudes, the effects of secondary messenger pathways, their components and local mediators generated as a direct result of mechanical load are known to result in an elevation of specific matrix protein mRNAs. Up-regulation of matrix protein production is paramount to tissue formation. Thus, mechano-transduction offers a method of producing bone tissue in vitro. However, successful transduction of mechanical stimuli from a substrate to cells is reliant upon a number of factors including cell–substrate adhesion, scaffold material mechanics and the activation of membrane channels, for example voltage-operated calcium channels (VOCC). Our research focuses on the optimisation of mechano-transduction pathways for successful bone tissue engineering. In this paper, we focus on the effects of cell–substrate adhesion, attenuation of VOCC activation states and biological conditioning of cell-scaffold constructs utilising bioreactors in relation to mechano-transduction-induced bone tissue production. The effects of these factors on successful bone tissue formation observed in increased matrix protein synthesis due to the optimisation of mechano-transduction pathways is discussed.

Résumé

L'ingénierie tissulaire offre de grandes perspectives par le traitement de pathologies osseuses ou le remplacement de tissus lésés lors de pertes massives de substances osseuses. Selon ce concept, un prélèvement de cellules prélevées chez le patient, amplifiées in vitre par des techniques de culture cellulaire, introduites dans un biomatériau bioactif, constitue ainsi le support de l'ingénierie tissulaire. Ces matériaux hybrides associant un biomatériau et des cellules autologues offrent une parfaite biosécurité pour le patient en éliminant tout risque de contamination ou de rejet lié à l'utilisation des allogreffes. Le mécanotransduction joue un rôle essentiel dans les mécanismes de remodelage osseux et de réparation tissulaire. Sur le plan physiologique, les contraintes mécaniques peuvent activer certaines voies de transduction du signal entraînant la production de seconds messagers et de différents médiateurs cellulaires. Ces événements ont pour conséquence de modifier l'activité transcriptionnelle de la cellule sous contrainte et modifier ainsi les taux des ARNn des protéines de la matrice extracellulaire. Au niveau cellulaire, ces mécanismes de mécanotransduction peuvent modifier la relation cellule/matériau et plus particulièrement l'adhésion cellulaire et activer des canaux membranaires tels que les canaux calciques voltage dépendant (VOCC). Dans ce travail, nos efforts se sont

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portés sur l'effet de contraintes mécaniques sur l'adhésion cellulaire à son support, sur l'activation des canaux calciques voltage dépendant et sur la production de matrice extracellulaire au sein d'un complexe hybride (biomatériau/cellule) cultivé en bioréacteur.

Keywords: Substrate; Bone cells; Adhesion; Mechanical loading; Matrix production

Mots clés : Materiau ; Cellules osseuses ; Adhésion ; Contraintes mécaniques ; Matrice extracellulaire

1. Introduction

One of the major challenges for tissue engineering is to create a favourable environment for the proliferation and differentiation of cells into functioning tissues. Three main elements compose engineered tissue, namely the scaffold, the cells and the environment in which the cell-scaffold constructs are cultured and conditioned. The design and function of synthetic scaffolds can be optimised, allowing for control of cell behaviour following seeding onto a final product. Numerous scaffold properties can be manipulated, including the type of material utilised, the shape and size of pores in which cells are located, the mechanical integrity of the construct, substrate coating aimed towards promotion of cell adhesion and incorporation of chemicals and growth factors conducive to optimised tissue formation and function. Following selection of a scaffold with desirable characteristics, cells can be seeded and tissue formed. Conditioning of cell-seeded constructs also allows for control and optimisation of tissue production and is achieved, in vitro, through the selection of an appropriate bioreactor. Bioreactors are universally used in tissue engineering to supply nutrients and dissolvable gases to cells contained within a porous three-dimensional scaffold. Following this, the selection of a bioreactor rests with the conditioning required for specific tissue production in a selected scaffold.

For many years biomechanical stimulation has been known as an essential stimulus for bone tissue remodelling and repair. Mechanical loading at physiologically-relevant magnitudes has been shown directly to initiate bone deposition and remodelling in animal models [7,27,36,42] and in organ culture [5,6,14,23,35]. In contrast, lack of load results in the promotion of tissue atrophy and bone loss [24,28]. In the case of connective tissues, the ability to function mechanically is key to successful implant tissue production. The ability of bone to sense its mechanical environment is coupled to the requirement of the cells responsible for tissue turnover to modulate their activity based on this information received. The role of these cells is ultimately to maintain or alter the nature of the bone structure depending on the strain placed upon the tissue. Furthermore, bone formation and remodelling around implanted materials is influenced by the loading regime. The nature of the implant material, its surface properties and the anatomical site of implantation are also key elements to successful implant integration.

Our aim is to control or enhance biomechanical cell signalling for improved production of engineered bone, ultimately resulting in improved implant integration. We have considered three approaches by which we can translate our understanding of cellular biomechanics in the field of bone tissue engineering: 1) design and choice of surface coatings which are optimal for cell adhesion and translation of load signals via a substrate; 2) development of 'mechano-active' scaffolds for tissue engineering, where a chemical agonist with controlled release is incorporated to attenuate VOCC activation times, increasing the mechanical response of cells; and 3) use of biomechanical conditioning for bone tissue constructs in bioreactors.

2. Modifying substrate surfaces to enhance mechano-transduction

Surface properties, for instance surface chemistry, hydrophilicity or surface tension and topography are known to influence cell–substrate interactions [1]. As the first interaction between cells and substrate is that of adhesion, the surface properties of a scaffold become a key factor in governing the successful outcome of engineered tissue. The attachment of cells to their support matrix is important in determining cell shape, and ultimately proliferation, and in maintaining proper cell function and tissue integrity [38]. Adhesions act to anchor cells to a substrate and provide positional signals that direct cellular traffic and differentiation [40]. Effective cell adhesion is required in bone tissue engineering, allowing the cascade of cellular events in a dynamic environment to occur, especially with respect to mechanical stimulation.

A number of events occur when a substrate surface is contacted by a biofluid, whether it is blood in vivo or media containing cells in vitro, resulting in a characterised material adopting a modified state which determines the final interactions between cells and a substrate [26]. Upon immersion in a biofluid, the material is hydrated with water molecules. Hydrated ions are then incorporated into the surface water layer, following which biomolecules, for example proteins, adsorb to the substrate. Thus, upon reaching a surface, cells are presented with an ionically screened and protein-coated substrate, which, in turn, has been determined by the initial characteristics of the material. As a result, cells do not attach directly to a substrate, for example glass or plastic, but to a layer of extracellular matrix proteins adsorbed to the surface from their surrounding biofluid.

For adhesive cell types in general and bone cells in particular, the role of cell-matrix/substrate interactions is central