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Recombinant Proteins: Hopes for Tissue Engineering

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SUMMARY

Proteins constitute a group of key molecules with many applications in tissue engineering. Use of proteins provided from natural sources has several limitations which are overcome by the use of recombinant proteins. So far, the recombinant forms of many proteins with tissue engineering applications have been developed including structural proteins, growth factors and cytokines. This technology has enabled the development of specifically designed proteins such as growth factors with matrix binding domains, and hybrid structural proteins with improved mechanical properties. Recombinant proteins are produced either *ex vivo* or *in vivo*, by local gene therapy protocols, and are of medical and economic benefits. Due to the high applicability of recombinant proteins in tissue engineering, it is recommended to include this platform as an infrastructural element in any tissue engineering program.

Tissue engineering is a developing field in medical and surgical therapeutics aiming at fabrication of biointeractive substitutes for functional restoration of lost tissues and impaired organs. Using three basic elements of cells, scaffolds and signaling molecules and depending on the complexity of the target tissue, different design methods and technologies may be applied to the engineering of a substitute. The three basic elements are known as the tissue engineering triad and could be inter-related by protein molecules. The proteins not only contribute to the structure of many signaling molecules and affect the cellular behavior, but also could be used for fabrication of scaffolds. Therefore, proteins constitute a group of key molecules that should be dealt with for many tissue engineering purposes. However, several limitations are implicit in the application of proteins including source availability and stability, batch-to-batch variability, transmission of infecting organisms and immunogenicity. These limitations have been overcome by the development of recombinant protein technology, which also has enabled the design of proteins with targeted properties.

As fibrous proteins have several cell and matrix functional domains (Sweeney *et al* 2008) and have excellent structural properties, they have been one of the integral components of synthetic scaffolds for a long time. However, sourcing of these proteins has imposed a major limit on their clinical use and encouraged the application of recombinant technology for their production. There are several successful reports of expression of proteins such as collagens (Wang *et al* 2008, Liu *et al* 2008), elastin (Jordan *et al* 2007, Sallach *et al* 2009), and spider silk (Rabotyagova *et al* 2009, Agapov *et al* 2009) in both prokaryotic and eukaryotic systems. These recombinant proteins can serve as invaluable safe and reproducible sources of structural proteins for fabrication of tissue engineering scaffolds. Although, these proteins naturally interact with both cellular and extracellular components of many tissues, their biological properties could be enhanced by further functionalization with other bioactive molecules such as growth factors or antibiotics. Functionalization is usually achieved by physical or chemical immobilization of the target molecules on the surface of the scaffold. However, recombinant technology enabled engineering of fusion proteins with bioactive properties and matrix binding domains. This approach leads to superior functionalization and higher stability of the product. The list of published fusion proteins is long, but FGF-1-collagen binding domain (Pang *et al* 2010) and VEGF-collagen binding domain (Yan *et al* 2010) could be referred to as prototype examples. Production of proteins containing additional functional domains such as silk-RGD (Kambe *et al* 2010), or production of antimicrobial peptides fused with a matrix binding domain or a structural protein (Gomes *et al* 2011) are other novel strategies to enhance reparative properties of the synthetic matrices. Additionally, fusion proteins could be designed for

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combining certain properties of different structural proteins. For example, a fusion protein made of silk and elastin (Qiu *et al* 2010) has been shown to possess the tensile strength of silk and the durability and resilience of elastin.

As it has been mentioned above, growth factors and cytokines are a group of proteins with important applications in tissue engineering. However, as the half-lives of these proteins are typically short, continuous or repeated delivery, often in high dosages, is necessary to ensure implementation of their desired action. This has been addressed by manual or pump delivery or by incorporation into the scaffolds (as described above) and design of a controlled release method. Manual or pump delivery, either *in vitro* or *in vivo*, is far from being optimal. For the controlled release, the applied chemistry may affect the potency of the protein. Therefore, some local gene therapy methods have been developed in order to use genetically modified cells which secrete the protein in question. Using transient or conditional transfection methods, the cells could be engineered to secrete the protein during a particular period of time. Although, several organs were included in this methodology, much of the work in this field has been performed for bone healing (Tang *et al* 2008, Peterson *et al* 2005).

Studies on the two FDA-approved growth factor-based bone substitutes INFUSE® (rhBMP-2, Medtronic, Minneapolis, MN) and OP-1™ (rhBMP-7, Stryker Biotech, Hopkinton, MA) have shown not only the clinical efficacy, but also the economic values of these products. For example, a detailed cost and benefit analysis of the surgical application of INFUSE® in UK, Germany and France has shown that despite the high direct cost of this product, it leads to a high net cost saving at the national level from a societal perspective. The saving is due to improvements in indicators such as return-to-work time, secondary interventions costs, and the overall treatment costs (Alt *et al* 2009). Same results are predicted for similar products. But, as the use of recombinant proteins face several challenges, the cost of development of these products is high. The main challenges that should overcome for most recombinant proteins are their short half-lives, development of immune response against proteins of non-human origin, and the need for post-translational modification of the protein. These hurdles preclude many of the recombinant proteins to be therapeutically effective and to clear regulatory approvals for clinical applications. However, there is a significant potential to address these hurdles when local gene therapy is considered as the means for delivery of recombinant proteins.

In conclusion, recombinant protein technology will highly enrich the field of tissue engineering and bring it closer to the clinical applications. Therefore, it is recommended to integrate this technology, as an infrastructural element, into any tissue engineering program (Samadikuchaksaraei 2007, Samadikuchaksaraei and Mousavizadeh 2008). The importance of such a platform needs a special emphasis, as most tissue engineering programs, in both developed and developing countries, do not take advantage of this platform.

Ethical issues

No ethical issues to be declared.

Conflict of interests

No conflict of interest to be declared.

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