THE ROLE OF BIOMATERIAL RESEARCH IN THE FIELD OF REGENERATIVE MEDICINE

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In its historical setting biomaterials science has been an applied science, as generally a clinical application is the deliverable which directs the research activity. However, in the age of "tissue engineering" (TE) and "regenerative medicine" (RegMed) it has become abundantly clear that this scientific endeavour contains numerous fundamental material and biological issues which equally qualify biomaterials science as a basic science. The paradigm change in our conception of "biocompatibility" from the absence of cytotoxicity to the demand for proof of biofunctionality has led many scientists worldwide to look at the structural and functional elements of interfaces in Nature. The "biomimetic concept" has been coined as an attractive term for the simulation of these natural functions. An holistic analysis of natural interfaces reveals two principal elements, namely cells, and their products, one important subgroup of which are matrix components (ECM : extracellular matrix). These considerations have led to different models for implant design for TE, one of the exciting models being that of a biodegradable matrix or scaffold which contains the essential bioactive signal molecules, for example as a drug- or gene-delivery system, to elicit a physiological regenerative response, in which colonizing cells would find adhesion promoting domains, as well as substrate domains to induce cell-driven resorption by enzyme secretion [1]. The degradation characteristics must be such that the biomaterial is present long enough to permit the necessary biological reactions to take place. Thus, future success of TE applications will depend on better understanding of cell-matrix interactions and the ability to regulate the cellular response at an implant interface, preferably by using synthetic or natural polymers which resemble ECM as closely as possible.

Although the use of a biomaterial to simulate the ECM will be a natural choice in many TE applications, it is probable that some concepts for TE and RegMed will not necessarily involve a biomaterial, this certainly being the case in pure cell therapies. Nevertheless, it is evident that research endeavour in TE necessitates a strong input from the material sciences on the one hand and the life sciences on the other. In the material sciences there have been major breakthroughs in the development of so-called "intelligent materials", that is, materials which are able to respond to microenvironmental stimuli, such as changes in temperature, pH, light wavelength and even cellular enzymes [2,3]. This offers the possibility to fine-tune a scaffold or matrix for application at specific sites, such as soft tissue or within bone. Advances in knowledge of both synthetic and natural polymers, including blends of the two sources, have opened up numerous opportunities for soft and hard tissue regeneration, although the problem of mechanical stability in load-bearing applications remains to be solved.

A further significant field is that of nanofabrication, with for example the use of self-assembly molecules and the possibility to create domain structures on the surface of biomaterials. The use of nanoparticles opens up new vistas for drug and gene delivery, although possible negative effects have promoted a new research area of "nanosafety". It is evident that strategies are required which permit an effective entry and targeting of the drug- and gene-delivery system (DDS and GDS resp.). Various possibilities can be conceived, depending on the tissue or organ to be targeted. Examples of biomaterial-associated DDS and GDS are coated stents for cardiovascular applications, nanoparticle targeting of the alveolo-capillary barrier in the lung and topical (skin) application of nanoparticulate systems.

In addition to the goal of designing and constructing a TE implant which is functional, a further goal must be to control the reaction of the body to the implant, or if it is an extracorporeal system, the effects of blood contact [4]. Thus, much more knowledge is required on how materials with different physical and chemical characteristics modulate the inflammatory and healing responses, as well as the specific phenotype of the cells essential to the application, such as chondrocytes and osteoblasts for cartilage and bone regeneration respectively. Central focal points of future research will be adult stem cell interactions with ECM-like scaffolds and matrices as well as deeper understanding of the signaling pathways involved. Immunological reactions at implant interfaces also remain inadequately investigated and how degradation products might elicit a specific immune reaction still remains virgin territory. Furthermore, the ability to promote rapid and physiological vascularization of TE implants will in most cases (with the exception of the anaerobic chondrocyte) determine the fate of the implant.

The complex nature of these interactions means that relevant models, both in vivo and in vitro need to be developed and extended to simulate the human in vivo state. The corollary of this is that, for example in in vitro testing schemes, more emphasis must be placed on the use of primary cells of human origin in assay systems which will yield useful data on cell functionality in interactions with biomaterials. This will involve three-dimensional culture systems, in which confocal laser scanning microscopy with relevant immunocytological methods can greatly assist monitoring functional parameters [5,6], as well as co-culture systems [7] and dynamic cultures, as in bioreactors. This increased level of complexity is regarded as essential for a deepening of our understanding of biological mechanisms, without which a rational approach to TE design will not be possible.

As the methods of genomics and proteomics become more and more applied to the field of TE, large data bases are being developed from cell culture as well as animal experimentation, this leading to the need to use the methods of systems biology to identify genes involved in the regulation of cell-scaffold interactions. It is hoped that the systems approach will further our understanding of these complex biological interactions and enable new more ECM-like scaffolds and matrices to be developed for regenerative medicine and tissue engineering. It is evident that in achieving this goal only the multidisciplinary approach will be successful.

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BIORESORBABLE HYDROGELS PREPARED FROM POLYLACTIDE/ POLY(ETHYLENE GLYCOL) BLOCK COPOLYMERS

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Introduction

The delivery of drugs to a human body can be achieved through oral, transdermal, topical and parenteral administrations. A great deal of work has been done during the past two decades to develop controlled drug delivery systems (DDS) adapted to these various routes.Hydrogels are of growing interest for applications as DDS because of their excellent biocompatibility due to the presence of large amounts of water [1-3]. Bioactive molecules can be physically entrapped in a hydrogel or chemically attached to the polymeric network. Hydrogels are usually formed by a hydrophilic polymer matrix crosslinked chemically through covalent bonds or physically through hydrogen bonds, crystallized domains or hydrophobic interactions.They are particularly interesting for the release of poorly soluble drugs, proteins, genes or nucleic acids [4-6].
In this work, polylactide/poly(ethylene glycol) block copolymers were prepared by ring opening polymerization of L- or D-lactide in the presence of PEG, using non toxic zinc powder as co-initiator. Hydrogels were prepared from aqueous solutions containing both PLLA/PEG and PDLA/PEG copolymers. The rheological properties and drug release behaviors of the hydrogels were investigated.

Experimental

Copolymers were synthesized by ring opening polymerization of L- or D-lactide in the presence of dihydroxyl PEG with Mn of 10000, 12000 and 20000 or monomethoxy poly(ethylene glycol) (mPEG) with Mn of 5000 by using zinc metal as catalyst.
Hydrogels were prepared by mixing predetermined amounts of PLLA/PEG and PDLA/PEG copolymers in 2 ml of distilled water. Gelation was allowed to proceed at predetermined temperatures for various periods of time. Hydrogels containing bovine serum albumin (BSA) were prepared under similar conditions, BSA being mixed in the aqueous solution before gelation.
In vitro release experiments were realized at 37°C by immersing 2 ml of BSA-containing hydrogel in 4 ml of phosphate buffered saline (PBS). The release was regularly monitored by U.V. at 277 nm, using calibration curves obtained from standard solutions.
H Nuclear magnetic resonance (NMR) spectra were recorded at room temperature with a Bruker spectrometer operating at 250 MHz by using DMSO-d_6 as solvent [1]. Rheological properties were determined on a Carri-Med CSL2 Rheometer of TA Instruments. The release of BSA was monitored by a Lambda 15 Perkin Elmer UV-Vis spectrophotometer. Circular dichroism (CD) spectra were registered with a Jobin Yvon CD6 instrument.

Results and discussion

Acronym	Structure	Initiator	Monomer	EO/LA	DP_{PEG}	DP_{PLA}	M_n
1L	$L_{19}EO_{227}L_{19}$	PEG10000	L-lactide	6.1	227	38	12700
1D	$D_{20}EO_{227}D_{20}$	PEG10000	D-lactide	5.6	227	40	13700
2L	$L_{20}EO_{273}L_{20}$	PEG12000	L-lactide	6.8	273	40	14900
2D	$D_{19}EO_{273}D_{19}$	PEG12000	D-lactide	7.3	273	38	14700
3L	$L_{21}EO_{454}L_{21}$	PEG20000	L-lactide	11.0	454	42	23000
3D	$D_{22}EO_{454}D_{22}$	PEG20000	D-lactide	10.5	454	44	23100
4L	$L_{28}EO_{113}$	mPEG5000	L-lactide	4.1	113	28	7000
4D	$D_{27}EO_{113}$	mPEG5000	D-lactide	4.2	113	27	6900

TABLE 1. PLA/PEG block copolymers obtained by ring opening polymerization of L(D)-lactide in the presence of PEG or mPEG

PLA-PEG-PLA triblock copolymers were synthesized by ring opening polymerization of L(D)-lactide in the presence of dihydroxyl PEG, while PLA-PEG diblock copolymers were synthesized by using mPEG5000 as initiator. Non-toxic Zn powder was used as catalyst instead of stannous octoate which can be more or less cytotoxic. TABLE 1 presents the molecular characteristics of the triblock and diblock copolymers used in this work. The molar ratio of ethylene oxide/ lactyl (EO/LA) repeating units was in the range of 4 to 11 for the water solubility of the copolymers.
FIG.1A shows the evolution of storage modulus (G') and loss modulus (G") of a 14% 1L/1D solution as a function of time at 25°C and at a frequency of 1 Hz. Initially, G" was higher than G', the solution behaving as a viscoelastic solution. Both G' and G" slightly decreased at first and remained constant during the first 60 min. Beyond, the moduli increased continuously, G' increasing faster than G". A crossover point was observed at 7 h. After that, G' became higher than G" and a hydrogel was formed. FIG.1B shows the changes of storage and loss moduli of the 14% 1L/1D sample as a function of frequency at t=0 and t=24h. Both moduli increased with increasing frequency. At t=0, the storage modulus G' was higher than the loss modulus G", and both moduli increased almost linearly with frequency, which is characteristic of a viscoelastic liquid-like state. In contrast, at t=24h, G' became higher than G", and both moduli tended towards a plateau at high frequency, which can be assigned to formation of a tridimensional network.
Bovine serum albumine (BSA) was retained as a model drug for release studies. The protein was mixed with the copolymer solution before gel formation. Various BSA-containing hydrogels were prepared under different gelation conditions in order to elucidate the drug release behaviors. Figure 2A shows the BSA release profiles of 20% 2L/2D hydrogels obtained after 90 h at 37°C or after 24 h at 50°C. The release rate appeared almost constant and there was almost