Bioactive biomaterials
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The most important advances in the field of biomaterials over the past few years have been in bioactive biomaterials. Materials have been developed to incorporate bioactivity through biological recognition, including incorporation of adhesion factors, polyanionic sites that mimic the electrostatics of biological regulatory polysaccharides, and cleavage sites for enzymes involved in cell migration. Materials have also been developed to be active in biological environments by undergoing phase changes in situ, including transformations from liquid precursors to solids and from soluble materials to materials that are immobilised on tissue surfaces.

Bioactivity by incorporation of adhesion factors

One approach toward biological activity in biomaterials is the incorporation of adhesion-promoting oligopeptides or oligosaccharides. Cell adhesion to traditional biomaterials, such as polyethylene, polytetrafluoroethylene or silicone rubber, is based upon indirect recognition, that is, by proteins from the body fluids adsorbing nonspecifically to the material surface and some subset of these adsorbed proteins, including fibronectin, fibrinogen, and vitronectin, promoting the adhesion of cells by interacting with the corresponding adhesion receptors. As a more direct approach, one that permits a greater degree of control by not depending upon a secondary mediator, several investigators have explored the covalent or physicochemical incorporation of adhesion-promoting oligopeptides and oligosaccharides. The reader may refer to other reviews on the molecules...
incorporated [1,2] and on methods that have been employed to incorporate them, in the context of biomaterials for tissue engineering [2-4] and drug delivery [5].

Extensive research has been performed on the incorporation of adhesion-promoting oligopeptides into biomaterial surfaces. These peptides are based on the primary structure of the receptor-binding domains of proteins such as fibronectin and laminin, and often the corresponding linear or cyclised sequences can display similar receptor specificity and binding affinity, as well as signalling of cellular responses, compared to the whole protein [1,2]. Early work demonstrated an important possible advantage of working with short adhesion peptides, rather than the complete parent protein, that is, that the peptides could be displayed in a manner such that nearly all of them were available and active for binding to cell-surface receptors [6]. When a bioactive tripeptide from fibronectin, the sequence RGD (amino acid single letter code), was immobilised by its amino-terminal primary amine via a glycyl spacer, approximately 10^5 copies per cell were required to induce cell adhesion, spreading, focal contact formation and cytoskeletal organisation, whereas many more copies of the complete protein were required, presumably due to unfolding of the protein associated with adsorption or absorption of the protein in an orientation such that the receptor-binding domain was not sterically available. Likewise, early work also demonstrated, in the example of the bioactive YIGSR pentapeptide from laminin, that peptides covalently immobilised in a single orientation could have considerably different biological activity than the same peptide adsorbed in a number of possible orientations, many of which were presumably sterically incapable of binding to their receptors [7].

There are several important fundamental issues about how such adhesion signals are displayed. For example, it has been demonstrated that cell adhesion strength depends upon the surface concentration of adhesion ligands, and furthermore, that cell migration rates depend in a very sensitive manner on the strength of cell adhesion [8**]. If cell adhesion strength is very low, the cell is incapable of developing adequate traction to migrate, whereas if cell adhesion strength is very high, this traction is also very high and the rate of reassociation of dissociated adhesion receptors is very fast, resulting in very slow migration. Thus, in situations where one would incorporate adhesion ligands to promote adhesion and migration (e.g. in the pores of a vascular graft to promote capillary ingrowth or in the pores of a wound healing membrane for promoting healing of skin ulcers) one must search for an optimum in cell migration with this phenomenon in mind. Furthermore, there may be cases in which the spatial display of adhesion ligands is critical. For example, in the immobilisation of galactose for promotion of hepatocyte adhesion via the asialoglycoprotein receptor, the degree of ligand mobility, as determined by the length of the flexible tether used for immobilisation, strongly influenced cell adhesion behaviour [9]. The extent of cell spreading depended on both the overall amount of ligand immobilised as well as its ability to cluster into spatial microdomains. This dependence is consistent with the binding of the receptor to triads of galactose residues, in a manner mimicking binding to the branched oligosaccharide in the natural ligand for the receptor.

What could be other advantages of employing short bioactive peptides or saccharides, rather than the complete parent glycoprotein? One goal is selectivity for targeted cell types. Cell-type selectivity is a common goal in drug targeting [5], and it may also represent an important goal in tissue engineering [2]. For example, in vascular graft design, it would be beneficial to develop a material to support the adhesion and migration of vascular endothelial cells, while at the same time rejecting the adhesion of blood platelets [10]. The integrin receptor αβ1 is a target that is present on the endothelial cell but not the blood platelet, and there exists an adhesion ligand specific to this receptor, the tetrapeptide REDV, in the adhesion protein fibronectin. Fibronectin does not provide a useful ligand for this purpose, however, because the protein contains numerous adhesion-promoting domains, at least one of which binds to most cells in the body. This lack of selectivity can be avoided by working with only the REDV domain, and not the other, less specific domains. Thus, one can accomplish a goal with a small domain of the protein that could not be accomplished with the complete parent proteins, that is, cell-type selectivity. Other goals in tissue engineering can also be obtained more easily with small peptides than with the whole proteins, such as the control of cell phenotype by exposure to certain adhesion ligands, as has been demonstrated in culture with bone-forming cells [11], and enhancement of cell adhesion strength, which has been shown to be important in clinical studies with endothelial cell seeded vascular grafts [12**].

Several biomaterial systems have been explored and developed for display of bioactive adhesion-promoting ligands, and a small number of these are discussed below to illustrate key points. More complete reviews on this topic can be found elsewhere [2,13]. Most investigators have approached the problem from the perspective of covalent immobilisation of adhesion-promoting ligands upon a biomaterial surface, usually after some type of surface modification [2,13], and this is usually adequate. For degradable materials, which are particularly useful in tissue engineering because they do not need to be removed from the body, surface modification may be inadequate because the surface can be rapidly degraded and removed. For such situations, it may be necessary to develop new polymers that display the ligand in the bulk of the material, and so display new ligands on the surface continually as it is degraded and remodelled. This has been addressed with the practically important degradable biomaterial polylactic acid by the synthesis of the copolymer polylactic acid-collidine). The ε-amino groups on the lysyl comonomer
provide sites of ligand grafting in solution, thus presenting the peptides on the transient surface as it moves through the extracellular matrix. Bulk incorporation is also useful in the case of gels that are subject to cell infiltration. Bioactive migration-promoting peptides, such as the YIGSR domain and the SIKVAV domain from laminin, have been incorporated within gels for use in nerve regeneration [15*]. In this case, the peptides were incorporated throughout the bulk of the three-dimensional gels to permit the infiltrating neuronal cells to contact the signals on all sides and at all times during the infiltration process.

Other approaches to the presentation of bioactive adhesion-promoting peptides have been to include the bioactive peptide sequences in the backbone of the polymer chains (e.g. in polypeptide biomaterials). For example, elastin-like polypeptides have been developed to produce elastic, protein-based biomaterials that contain adhesion sites, such as the RGD tripeptide, engineered within the polypeptide sequence [16].

**Bioactivity by incorporation of growth factors**

Polypeptide growth factors are powerful regulators of a variety of cellular behaviours, including cell proliferation, migration, differentiation, and protein expression, and these molecules are being developed as important therapeutics in tissue regeneration (e.g. in closing bone defects and in healing chronic ulcers in the skin). Growth factors are also being explored as key components of biomaterials and biomaterial systems, as discussed in the illustrative examples below. Why is it useful to consider growth factors as part of a system involving biomaterials, rather than on their own? The biological activity of the growth factor depends not only upon its identity, but also upon how it is presented to the cells in space and over time. For example, it has been demonstrated that some growth factors are more effective when provided to cells through a controlled release process, whereas others are more effective when presented as a bolus [17]. This difference in behaviour may be related to how the cells traffic and recycle their receptors for these growth factors, and it may be possible to modulate trafficking and recycling by altering either the growth factor or its interactions with a biomaterial that is releasing or presenting it. Under the natural conditions of the body, the extracellular matrix plays a role in storing, displaying and releasing growth factors. Given that the natural biomaterial of the body plays this important role, it seems reasonable to explore this and related behaviour with biomaterials, to mimic this function of the extracellular matrix.

Controlled release systems have been developed for growth factors, for example, based on traditional biomaterials in delivering angiogenic growth factors in vascular repair [18] or in delivering neuronal survival and differentiation factors in neurodegenerative diseases [19]. Furthermore, specialised biomaterials have been developed to incorporate and modulate these important biologically active molecules. For example, with the goal of bone repair by delivery of the growth factor transforming growth factor β, the affinity of the growth factor for heparin has been exploited [20••]. Many such growth factors bind heparin, as well as heparin sulphate proteoglycans in the extracellular matrix. To exploit this binding affinity, heparin was conjugated to collagen matrices used in bone repair, and this immobilised heparin served as an affinity site to bind and slowly release the growth factor in the healing site. As an extension of this approach, the growth factor was chemically conjugated to the collagen matrix via a poly(ethylene glycol) spacer. This growth factor was biologically active in the immobilised state, presumably being able to bind to the receptors on the surface of the cell and permit receptor dimerisation and signal transduction [21••]. The ability of immobilised growth factors to be biologically active has also been demonstrated in the very well-characterised system of epidermal growth factor conjugated to synthetic polymer surfaces, where it was shown to be capable of directing hepatocytes to maintain their liver-specific morphology and function [22••].

Exciting advances have been made with the display and delivery of precursors for polypeptide growth factors, as well as the growth factors themselves. It has been demonstrated recently that plasmid DNA can be efficiently taken up and the encoded gene expressed when the plasmid is presented on the surface of a biomaterial. This has been shown in the context of smooth and cardiac muscle cells by presentation of plasmid DNA on sutures [23••], as well as in the context of bone repair, in which fibroblasts in the repair tissue took up the plasmid DNA encoding the growth factor bone morphogenetic protein-4, and by expression of the growth factor enhanced bone formation [24••]. Cellular and DNA interactions with the biomaterial appear to be important in determining the extent of uptake by the cells in the repair site; as such, the DNA-presenting biomaterial surface becomes a bioactive biomaterial that expresses its bioactivity for long periods of time by affecting the behaviour of the cells that come in to contact with the biomaterial.

**Bioactivity by physicochemically based biological recognition**

Some biological interactions are based on physicochemical interactions that are less specific than those described in the previous sections, such as adsorption due to electrostatic interactions, which can be readily mimicked and incorporated into bioactive biomaterials. Heparin’s electrostatically dominated interactions in anticoagulation present one example, and extensive work has been performed at immobilisation of heparin on biomaterials to render them bioactive [25]. Extensive work has also been performed to obtain other polymers that possess heparin-like activity, for example, by screening dextrans with varying extents of sulphonation [26,27] or by identification...
of plant polysaccharides with a fortuitously appropriate charge density and structure, as in the example of fucan [28]. Such biological activity can be extended beyond the anticoagulant activity of heparin (i.e. binding to the proteins antithrombin III and thrombin to catalyse complex formation between these two proteins), to other activities of heparin, such as binding to growth factors or interfering with a growth factor's binding to its receptor [29]. It is interesting to note that similar biological activity can be obtained completely outside the platform of a polysaccharide chain (as seen with heparin) for charge presentation. For example, with appropriate degrees of sulfonation, polyurethanes can function as heparin-like water-soluble polymers [30].

Novel biomaterials for biosensing have recently been developed by combination of less specific activity based on physicochemical interactions and highly specific activity based on enzymatic activity. Synthetic polymer hydrogels have been developed as three-dimensional conductors, with redox active charge transfer centres chemically incorporated within the gel network [31••,32••]. Redox enzymes, such as glucose oxidase, can be incorporated and even chemically bonded throughout the network to provide for efficient electron transfer from the enzyme’s active site, through the gel by transfer from site to site, to a collection electrode to form a concentration-dependent sensor for glucose. Thus, careful combination of individual biomaterial properties, such as polymer chain and network dynamics (which controls charge transfer through the gel by modulating transient close approaches to within electron tunnelling distances between immobilised charge transfer sites) and enzyme coupling (which controls charge transfer from the enzyme to the gel), can lead to a biomaterial with a highly specialised and selective biological activity.

**Bioactivity by incorporation of enzymatic recognition sites**

The two sections above, dealing with incorporated adhesion and growth factors, addressed the transmission of biological information from a biomaterial to the neighbouring cells. One can also consider the other direction, in which the biomaterial is the recipient of information produced by cells. One such form of information is enzymatic activity associated with the cell surface during cell migration. Cell migration through collagen [33] and fibrin [34] gels, both natural biomaterials involved in the generation, remodelling and regeneration of tissues, has been explored and is known to depend mainly upon the sensitivity of the material to proteases produced by the cells, upon the amount of enzyme produced by the cells, and upon the amount of material to be remodelled by the cells as they migrate through the material.

Approaches have been developed to engineer biomaterials that can be remodelled by cells through cell-associated enzymatic activity. Cells naturally remodel the extracellular matrix in development, adaptation and healing, and materials that are subject to the remodelling activities of the cell may enable exploitation of these biological activities in tissue engineering. For example, a fascicle route to the chemical incorporation of bioactive signals has been developed for fibrin, a natural biomaterial matrix that can be remodelled proteolytically. In this modification scheme, exogenous peptides bear in one domain a substrate for the transglutaminase involved in coagulation, factor XIIIa, and are thus covalently conjugated to the fibrin network as it forms, incorporating the bioactive peptide within the gel. Another domain of the peptide bears a bioactive peptide, for example, with cell adhesion or growth factor binding activity [35••]. Through such a route, it is possible to incorporate the biological activity of a host of non-fibrin proteins (e.g. laminin) as synthetic components added into the platform of the biologically-derived fibrin gel.

Completely synthetic biomaterials have been designed that are proteolytically degradable and that comprise other bioactive components as well. Gels have been formed based on poly(ethylene glycol) chains comprising central oligopeptides, sites that are substrates for collagenase or plasmin, both of which are involved in cell migration [36••]. These water-soluble hybrid chains may then be coupled at their termini to form three-dimensional elastic gels that are completely synthetic, but which are also degradable by cell-associated enzymatic activity. Additional biological activity can be conferred upon the proteolytically remodable gels by copolymerisation of suitably reactive oligopeptides, such as terminally reactive poly(ethylene glycol) grafted with the adhesion peptide RGD [37]. It is thus possible with these approaches to construct materials that possess a large number of the characteristics of the natural extracellular matrix, but which are totally synthetic and capable of fascicle manufacture and customisation.

**Bioactivity by material transformation**

Biomaterials can possess biological activity (i.e. activity in a biologically relevant context) via the ability to be transformed from one state to another. Several examples have already appeared and some have already found clinical utility, and selected examples are presented below. The reader is directed elsewhere for a review exclusively on this topic [38].

Materials that undergo phase transformations have a great deal of potential for use in surgery, for example, as adhesives, sealants, and barriers to cell–tissue contact. A water-soluble macromer has been developed based on poly(ethylene glycol) central blocks with oligo(lactic acid) flanking blocks and terminal acrylates; the large central block provides water solubility, the flanking oligoester degradability, and the terminal sites of unsaturation polymerisability [39]. These soluble materials can be rapidly transformed into elastic hydrogels by exposure to light in the presence of suitable photoinitiators, such as eosin yellowish. These materials have been employed to block blood platelet adhesion to blood-vessel surfaces after
injury to thereby improve the postsurgical healing of the vessel [37,40], and to serve as a barrier and depot for local drug delivery to prevent the formation of scar tissue adhesions between organs after gynaecological surgery [41]. Photocurable, and photolysable, hydrogels have also been developed based on cinnamylidene acetate dimerisation, rather than acrylate polymerisation as in the examples above [42,43]. Materials that can be converted from liquids into elastic gels by reaction after mixing of two liquids have also been developed, for example, by reaction of N-hydroxysuccinimidy activated esters on the end of a difunctional poly(ethylene glycol) with amines on the end of a tetrafunctional poly(ethylene glycol) [44]. This scheme has also been employed to synthesise enzymatically degradable gels, by using an aminated hyaluronic acid chain as one component of the two-liquid system (D Aeschlimann, personal communication). All of the reactions described above (i.e. gel formation by photoinitiation or by mixing of two liquids) can be sufficiently gentle to be carried out in vivo. For example, photopolymerisation of degradable poly(ethylene glycol) acrylates is currently used to seal leaking tissue surfaces after surgery in man.

A second sort of material transformation that can be used to modulate biological interactions is gel swelling or collapse in response to temperature changes. Material chemistries that can be employed to lead to polymers displaying lower critical solution behaviour (giving gels on warming of liquids) or upper critical solution behaviour (giving gels on cooling of liquids) have been described [45]. Such materials have been used as injectable depots for drug delivery (after a liquid-to-solid transformation) [46], as reversible cell culture substrates upon which cell monolayers and multilayers can be cultured and then released intact for transplantation (after a hydrophobic-to-hydrophilic transformation) [47], and to block a ligand-binding site on a protein in a thermally regulatable manner (after an extended-chain-to-collapsed-chain transformation) [48,49]. Such transformations can also be obtained in protein solutions, also based on changes in temperature, as well as changes in salt concentration. For example, self-assembly can be obtained in protein based on silk and elastin to form gels [50], as well as by electrostatic interactions in other designed oligopeptides and polypeptides employing leucine zipper domains [51] and ionic self-complementary domains [52].

As a final example of approaches toward bioactivity through material transformation, schemes to develop biomaterial monolayers as therapeutics have been developed. In the examples on photopolymerisable hydrophilic macromers discussed above, a therapeutic goal was blockade of cell interactions with a tissue surface after injury associated with surgery. This might also be obtainable with polymer monolayers, only nanometers thick, rather than gels tens of microns thick. This goal has been approached by the design of multifunctional polymers bearing domains that bind to tissue surfaces based on electrostatic interactions via a backbone of poly(lysine), in addition to other domains that repel the binding of proteins and cells via pendant blocks of poly(ethylene oxide) [53••,54]. These graft co-polymers were demonstrated to bind to complex biological surfaces, through affinity for their net-negative charge, and sterically stabilise them against subsequent adhesion, such as preventing lectin-induced agglutination of red blood cells [53••] or preventing the formation of postoperative adhesions [54]. Such approaches of assembly on biological surfaces have been taken even further, to actually chemically graft amine reactive poly(ethylene glycol) derivatives to lysine residues in proteins on tissue surfaces to inhibit cell adhesive interactions after surgical tissue damage, where such chemical grafting is performed under conditions sufficiently mild to permit grafting in vivo [55].

Conclusions

Biological activity has played an important role in modern biomaterials development, employing the principles of biological recognition that are used so frequently in pharmaceutical design in addition to other more material-centric principles, for example, those that permit material to respond to external stimuli. Only recently have these novel bioactive biomaterials begun to make clinical impact, but given the relatively long cycle from concept to clinic this is to be expected. Indeed, it is probable that the concepts of bioactivity reviewed here, as well as others, will make much more direct clinical impact in the culture laboratory and clinic in the next few years. It is clear that the expenses associated with development and regulatory approval of products based on bioactive biomaterials will be higher than that of products based on traditional biomaterials, and as such these development activities must be targeted at economically important applications with largely unmet needs, where advantages in safety and efficacy associated with bioactivity compensate favourably for the higher cost of development and regulatory approval of the bioactive product.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
**of outstanding interest


This paper provides very convincing evidence of a direct link between cell adhesion strength and cell migration rates, providing for the biologists engineer important guidance that too much of a good thing (e.g., an adhesion ligand) can be bad (e.g., if cell migration was an important performance criterion).


This report, along with [21••], provides evidence that covalently immobilized growth factor, in this case epidermal growth factor, can retain its biological activity. In addition to the points made to [21••], this demonstrates that internalisation of the ligand-bound receptors is not critical in order to express biological activity.


This clinical study with endothelial cell seeded vascular grafts demonstrated that graft performance in high flow regions (where stresses on the attached endothelial cells were greatest) was better when the biomaterial matrix included the adhesion protein fibronectin. Although the study was performed with a complete protein, rather than an engineered peptide, it provides direct clinical evidence of the need, and probability of success, of such approaches.


The investigators employed grafted adhesion peptides from laminin to enhance the rate of neurite extension through a three-dimensional gel to which the peptide was attached. This provides an example of peptide grafting for heparin to develop clinically relevant antiproliferative activity, binding and internalization. Eur J Cell Biol 1997, 74:376-384.


28. Logeart D, Prigent-Richard S, Jozefowicz J, Crepin M: Characterization and development of a glucose concentration into an amperometric signal. This permits amplification of the bioactivity associated with the biomaterial surface, in that the cells that come into contact with the material can continue to express the gene encoded on the plasmid DNA for an extended period of time.


This pair of reports (with [24••]) demonstrates that plasmid DNA, delivered upon a biomaterial surface, can be efficiently taken up by cells and the encoded gene expressed in a wound healing environment. This was demonstrated to have clinical relevance in repair of skeletal and cardiac muscle [23••] and in bone [24••]. This permits amplification of the bioactivity associated with the biomaterial surface, in that the cells that come into contact with the material can continue to express the gene encoded on the plasmid DNA for an extended period of time.


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This pair of papers (with [32••]) demonstrates that redox enzymes can be chemically conjugated into conducting polymer gels to obtain efficient electron transfer from the enzyme’s active site to the gel and ultimately to a collecting electrode. This permitted extremely highly sensitive and fast transduction of a glucose concentration into an amperometric signal. This high level of bioactivity was obtained by combining several features of bioactivity in a single material.


Fibrin is a very important biomaterial, both in natural wound healing and as a therapeutic. It possesses only one set of biological signals, and thus obtains generally only one sort of healing response, namely a scar. The investigators present a simple approach by which to functionalise fibrin with exogenous
bioactive peptide signals, by using bi-domain peptides, one peptide containing a substrate site for the coagulation transglutaminase factor XIIIa and the other containing a bioactive peptide of interest. This opens a fascicle route to make fibrin clots with customised biological activity.


This report, although preliminary in nature, provides an example of a totally synthetic biomaterial gel that can be degraded by cell-associated proteases. Given that adhesion ligands can also be incorporated into this material [37], this opens the door for synthetic materials that can be invaded and remodelled by cells in a healing response, in complete analogy with the way tissues are naturally remodelled.


