Biomaterial characteristics important to skeletal tissue engineering

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Tissue engineering has been investigated as a potential method for healing traumatized tissue. Many tissue engineering strategies utilize in vitro cell-scaffold composites formed in bioreactor systems and latter used for in vivo transplantation. A pre-requisite for in vitro tissue engineering is to grow sufficient cells on the scaffold. Furthermore, it is crucial to induce cells to express phenotypic characteristics of the target traumatized tissue. This is particularly important when using stem cells in tissue engineering, as the multipotent nature of stem cells is influenced by the growth environment. Thus, cell adhesion, proliferation, and phenotypic differentiation on scaffold materials are important considerations in tissue engineering.

Successful tissue engineering is strongly dependent on cell-biomaterial interactions. Cell adhesion to biomaterials is the first such interaction and the quality of this phase influences further cell functions. Cells sense chemical and physical signals from the extracellular matrix (ECM) and scaffolding material at focal contacts via transmembrane proteins (i.e., integrins) and shared within cell ensembles via cell-cell adhesion proteins (i.e., cadherins) and cell-cell communications (i.e., gap junctions). These signal transduction processes significantly influence cell growth and differentiation. Such signal transmitting structures, e.g., integrins-linker proteins-cytoskeleton, may be altered depending on biomaterial characteristics. In parallel, cell attachment and proliferation rate substantially vary depending on biomaterial characteristics, which also influences intercellular interactions. Both aspects potentially influence cell differentiation. Thus, scaffold biomaterials are an important parameter in both cell growth and differentiation.

Considerable progress has been made in tissue engineering of various tissues including bone, cartilage, blood vessel, liver, etc. However, scaffold materials have yet to be optimized with respect to surface characteristics (chemistry, topography, surface energy, etc.) and three-dimensional scaffold morphology (porosity, pore size, pore connectivity, etc.). In skeletal tissue engineering of bone and cartilage, an understanding of the effect of material characteristics is of crucial importance as it has been reported that osteoblasts and chondrocytes are sensitive to subtle differences in material chemistry, wettability, and roughness. We will highlight the effects of well-defined biomaterial characteristics on bone cell behavior and suggest future research topics on cell-material interactions for the development of optimal biomaterials.

The wettability of biomaterials is of primary concern as all in vitro and in vivo cell growth environments in tissue engineering are based on the wetting of biomaterials by liquid-like substances, e.g., media and body fluid. The wetting of biomaterials is influenced by various factors. Surface energy of the biomaterial, which varies as a function of intrinsic chemical composition and net polarity, strongly influences wettability. Surface energy is to a solid what surface tension is to a liquid, and both have a unit of J/m² (energy concept) or N/m (mechanical concept). If the surface energy of a biomaterial is higher than the surface tension of a liquid, the liquid will form a droplet with high contact angle and the substrate is ‘hydrophobic’. For the reverse case, the liquid will form a droplet with high contact angle and the substrate is ‘hydrophilic’. Besides, viscosity of the liquid and surface roughness of the solid influence wettability. Water adhesion tension (τ) calculated by water contact angle (θ) is often used as a measure of water wettability (τ = γw/v cosθ, where γw = 72.8 dyne/cm is water surface tension).

Cell adhesion is generally enhanced on hydrophilic surfaces. To reveal the osteoblast response to the entire biomaterial wettability window, we produced model substrata with various water wettabilities covering the wettability range within...
which most scaffolding biomaterials fall (0° < θ < 120°)21. We observed that human fetal osteoblastic (hFOB 1.19, hFOB) cells, a model for osteoprogenitor cells, exhibited a strong, positive correlation in cell adhesion and proliferation with substratum surface wettability22. hFOB cells displayed greater adhesion and proliferation on hydrophilic surfaces (30° < τ < 73 dyne/cm) than on hydrophobic surfaces (-40° < τ < 30 dyne/cm). Cell adhesion and proliferation revealed a transition behavior at the hydrophilic/hydrophobic boundary of ca. τ = 30 dyne/cm (θ = 65°). Biodegradable polymers including Polyactide (PLA) and Poly(lactide-co-glycolide) (PLGA), both FDA-approved scaffolding biomaterials, were relatively hydrophobic (θ ~ 70°) and followed the general continuum trend line in the adhesion-τ plot. Thus, biodegradable polymers were mostly in the transition boundary and small changes in wettability resulted in relatively large differences in cell adhesion.

Focal adhesion structure development depending on biomaterial characteristics per se, independent of ECM proteins, has not been widely investigated29,30. Primary human osteoblasts cultured on titanium and cobalt-chrome exhibited a notable absence of α5, α3, and αv integrin subunits36. Integrin α5, α3, and β3 expression by primary human osteoblasts was greater on PLGA than on PLA30. These results strongly suggest that osteoblast adhesion to biomaterials is mediated by differential integrin expression profiles. In a similar context but using a more systematic approach, we found that the development of focal adhesion assembly in osteoblastic cells was differentially regulated by substratum wettability22. Among the integrin subunits (α5, α3, αv, α5, β1, and β3) examined, hFOB cells cultured on poorly-wettable substrata (θ ~ 20°) expressed significantly lower αv and β3 than cells on fully-wettable substrata (θ = 0°) as assessed by Western blot analysis. Vinculin mimicked variations of αv and β3. In immunofluorescence assays, hFOB cells cultured on hydrophilic surfaces displayed distinct plaques of αv and β3 subunits, αvβ3 bound to one another, and vinculin, as well as their co-localization with well-developed actin stress fiber ends23,31. However, cells on hydrophobic surfaces displayed diffused integrin αvβ3, vinculin, and actin immunoreactivity. Therefore, actin cytoskeletons are anchored and tensioned at focal contacts composed of integrin αvβ3 and vinculin when hFOB cells adhere to biomaterials. The development of these signal transmitting structures is significantly improved on hydrophilic surfaces. On the other hand, hFOB cells grown on hydrophobic substrata, with downregulated αv and β3 expression, displayed higher steady-state osteopontin (OP) mRNA levels than cells on hydrophilic substrata as assessed by real-time RT-PCR analysis. An intriguing possibility is that cells, by upregulating OP, an ECM protein having Arg-Gly-Asp (RGD) integrin recognition sequences32, are trying to condition the environment for optimal cell growth. Steady-state mRNA levels of type I collagen, which binds to integrin αvβ3,33, did not show significant variation with substratum wettability, as was also the case for α5 and β3 integrin subunits.

We have demonstrated that biomaterial wettability can affect not only osteoblastic cell adhesion and proliferation but also focal adhesion structure development. Surface wetting also interferes with protein adsorption, not only in the kind and amount but also in conformational changes of adsorbed proteins, which will in turn affect cell adhesion34. Thus, cells may be affected both directly from biomaterial wettability and indirectly via altered protein adsorption35. Similarly, biomaterial surface roughness may affect cell functions both directly and indirectly via interfering protein adsorption. Additionally, surface roughness has a secondary effect of influencing surface wettability27,36.

In comparison with distinct, systematic early-stage effects of biomaterial characteristics on bone cell behavior, their long-term effects on bone cell differentiation are not as well understood. One interesting, current topic in tissue engineering is whether biomaterials per se or ECM-modified biomaterials accelerate stem cell differentiation into specific lineages. It was recently reported that cell shape can regulate commitment of human mesenchymal stem cells (hMSCs) to adipocyte or osteoblast lineage by allowing selective hMSC differentiation on patterned fibronectin surfaces with various size islands36. Another interesting subject in skeletal tissue engineering is mechanosensitivity of bone or stem cells37. One question to focus on is whether these cells respond differently to physical stimuli, including substrate deformation and fluid flow, when they are grown on biomaterials with varying characteristics, thus displaying different signal transmitting structures.

In conclusion, optimal bone forming cell-biomaterial interaction has not yet been developed for tissue engineering purposes and very little is known of biomaterial interactions with stem cells. Thus, further studies are required in this area especially as regards stem cell differentiation and mechanotransduction on different biomaterials with varying material characteristics.

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References