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Perspective Article

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[1]. 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[2]. 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[3]. Cells sense chemical and physical signals from the extracellular matrix (ECM) and scaffolding material at focal contacts via transmembrane proteins (i.e., integrins)[4-7]. These signals are further transmitted to the cytosol and nucleus via linker proteins (i.e., vinculin, talin, paxillin) and cytoskeletal proteins (i.e., actin)[8] and shared within cell ensembles via cell-cell adhesion proteins (i.e., cadherins)[9] and cell-cell communications (i.e., gap junctions)[10]. These signal transduction processes significantly influence cell growth and differentiation[11-14]. 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.[15-21]. 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.)[22,23]. 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[3,22,24-26]. 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[27]. 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/m^2 (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 becomes wettable on the substrate displaying low contact angle and the substrate is 'hydrophilic'. For the reverse case, the liquid will form a droplet with high contact angle and the substrate is 'hydrophobic'. 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 ($\tau = \gamma_{lv} \cos\theta$, where γ_{lv} = 72.8 dyne/cm is water surface tension)[28].

Cell adhesion is generally enhanced on hydrophilic surfaces[3,22]. To reveal the osteoblast response to the entire biomaterial wettability window, we produced model substrata with various water wettabilities covering the wettability range within

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which most scaffolding biomaterials fall ($0^o < \theta < 120^o$)[24]. 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 wettability[24]. hFOB cells displayed greater adhesion and proliferation on hydrophilic surfaces ($30 < \tau < 73$ dyne/cm) than on hydrophobic surfaces ($-40 < \tau < 30$ dyne/cm). Cell adhesion and proliferation revealed a transition behavior at the hydrophilic/hydrophobic boundary of ca. $\tau = 30$ dyne/cm ($\theta = 65^o$). Biodegradable polymers including Polylactide (PLA) and Poly(lactide-co-glycolide) (PLGA), both FDA-approved scaffolding biomaterials, were relatively hydrophobic ($\theta \sim 70^o$) 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 investigated[29,30]. Primary human osteoblasts cultured on titanium and cobalt-chrome exhibited a notable absence of α_3, α_5, and α_6 integrin subunits[29]. Integrin α_2, α_5, and β_1 expression by primary human osteoblasts was greater on PLGA than on PLA[30]. 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 wettability[25]. Among the integrin subunits (α_2, α_3, α_4, α_5, α_v, β_1, and β_3) examined, hFOB cells cultured on poorly-wettable substrata ($\theta \sim 120^o$) expressed significantly lower α_v and β_3 than cells on fully-wettable substrata ($\theta = 0^o$) as assessed by Western blot analysis. Vinculin mimicked variations of α_v and β_3. In immunofluorescence assays, hFOB cells cultured on hydrophilic surfaces displayed distinct plaques of α_v and β_3 subunits, $\alpha_v\beta_3$ bound to one another, and vinculin, as well as their co-localization with well-developed actin stress fiber ends[25,31]. However, cells on hydrophobic surfaces displayed diffused integrin $\alpha_v\beta_3$, vinculin, and actin immunoreactivity. Therefore, actin cytoskeletons are anchored and tensioned at focal contacts composed of integrin $\alpha_v\beta_3$ 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 α_v and β_3 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 sequences[32], are trying to condition the environment for optimal cell growth. Steady-state mRNA levels of type I collagen, which binds to integrin $\alpha_2\beta_1$[33], did not show significant variation with substratum wettability, as was also the case for α_2 and β_1 integrin subunits.

We have demonstrated that biomaterial wettability can affect not only osteoblastic cell adhesion and proliferation but also focal adhesion structure development. Surface wettability 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 adhesion[34]. Thus, cells may be affected both directly from biomaterial wettability and indirectly via altered protein adsorption[35]. 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 wettability[27,35].

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 islands[36]. Another interesting subject in skeletal tissue engineering is mechanosensitivity of bone or stem cells[37]. 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

1. Langer R, Vacanti JP. Tissue engineering. Science 1993; 260:920-926.
2. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284:143-147.
3. Anselme K. Osteoblast adhesion on biomaterials, Biomaterials 2000; 21:667-681.
4. Hynes RO. Integrins: bidirectional, allosteric signaling machines. Cell 2002; 110:673-687.
5. Hynes RO. A re-evaluation of integrins as regulators of angiogenesis. Nat Med 2002; 8:918-921.
6. Alenghat FJ, Ingber DE. Mechanotransduction: all signals point to cytoskeleton, matrix, and integrins. Sci STKE 2002; 119:PE6.

7. Ginsberg MH, Du X, Plow EF. Inside-out integrin signaling. Curr Opin Cell Biol 1992; 4:766-771.
8. Chen CS, Alonso JL, Ostuni E, Whitesides GM, Ingber DE. Cell shape provides global control of focal adhesion assembly. Biochem Biophys Res Commun 2003; 307:355-361.
9. Yap AS, Kovacs EM. Direct cadherin-activated cell signaling: a view from the plasma membrane. J Cell Biol 2003; 160:11-16.
10. Donahue HJ. Gap junctions and biophysical regulation of bone cell differentiation. Bone 2000; 26:417-422.
11. Yamada KM, Miyamoto S. Integrin transmembrane signaling and cytoskeletal control. Curr Opin Cell Biol 1995; 7:681-689.
12. Schwartz MA, Schaller MD, Ginsberg MH. Integrins: emerging paradigms of signal transduction. Annu Rev Cell Dev Biol 1995; 11:549-599.
13. Meredith JE Jr, Winitz S, Lewis JM, Hess S, Ren XD, Renshaw MW, Schwartz MA. The regulation of growth and intracellular signaling by integrins. Endocr Rev 1996; 17:207-220.
14. Giancotti FG, Ruoslahti E. Integrin signaling. Science 1999; 285:1028-1032.
15. Vacanti CA, Upton J. Tissue-engineered morphogenesis of cartilage and bone by means of cell transplantation using synthetic biodegradable polymer matrices. Clin Plast Surg 1994; 21:445-462.
16. Sikavitsas VI, Bancroft GN, Mikos AG. Formation of three-dimensional cell/polymer constructs for bone tissue engineering in a spinner flask and a rotating wall vessel bioreactor. J Biomed Mater Res 2002; 62:136-148.
17. Abukawa H, Terai H, Hannouche D, Vacanti JP, Kaban LB, Troulis MJ. Formation of a mandibular condyle *in vitro* by tissue engineering. J Oral Maxillofac Surg 2003; 61:94-100.
18. Nerem RM, Seliktar D. Vascular tissue engineering. Annu Rev Biomed Eng 2001; 3:225-243.
19. Nasseri BA, Pomerantseva I, Kaazempur-Mofrad MR, Sutherland FW, Perry T, Ochoa E, Thompson CA, Mayer JE Jr, Oesterle SN, Vacanti JP. Dynamic rotational seeding and cell culture system for vascular tube formation. Tissue Eng 2003; 9:291-299.
20. Jiang JL, Kojima N, Kinoshita T, Miyajima A, Yan W, Sakai Y. Cultivation of fetal liver cells in a three-dimensional poly-L-lactic acid scaffold in the presence of oncostatin M. Cell Transplant 2002; 11:403-406.
21. Li RK, Jia ZQ, Weisel RD, Mickle DA, Choi A, Yau TM. Survival and function of bioengineered cardiac grafts. Circulation 1999; 100:63-69.
22. Boyan BD, Hummert TW, Dean DD, Schwartz Z. Role of material surfaces in regulating bone and cartilage cell response. Biomaterials 1996; 17:137-146.
23. Murphy WL, Dennis RG, Kileny JL, Mooney DJ. Salt fusion: an approach to improve pore interconnectivity within tissue engineering scaffolds. Tissue Eng 2002; 8:43-52.
24. Lim JY, Liu X, Vogler EA, Donahue HJ. Systematic variation in osteoblast adhesion and phenotype with substratum surface characteristics. J Biomed Mater Res 2004; 68A:504-512.
25. Lim JY, Taylor AF, Li Z, Vogler EA, Donahue HJ. Integrin expression and osteopontin regulation in human fetal osteoblastic cells mediated by substratum surface characteristics. Tissue Eng 2005; in press.
26. Ishaug-Riley SL, Okun LE, Prado G, Applegate MA, Ratcliffe A. Human articular chondrocyte adhesion and proliferation on synthetic biodegradable polymer films. Biomaterials 1999; 20:2245-2256.
27. Al-Omari WM, Mitchell CA, Cunningham JL. Surface roughness and wettability of enamel and dentine surfaces prepared with different dental burs. J Oral Rehabil 2001; 28:645-650.
28. Vogler EA. Water and the acute biological response to surfaces. J Biomater Sci Polym Ed 1999; 10:1015-1045.
29. Sinha RK, Tuan RS. Regulation of human osteoblasts integrin expression by orthopaedic implant materials. Bone 1996; 18:451-457.
30. El-Amin SF, Attawia M, Lu HH, Shah AK, Chang R, Hickok NJ, Tuan RS, Laurencin CT. Integrin expression by human osteoblasts cultured on degradable polymeric materials applicable for tissue engineered bone. J Orthop Res 2002; 20:20-28.
31. Lim JY, Vogler EA, Donahue HJ. Integrin expression and osteopontin regulation in human fetal osteoblastic cells mediated by substratum surface characteristics, 50th ORS, p43, March 7-10, 2004, San Francisco, California, USA.
32. Liu YK, Uemura T, Nemoto A, Yabe T, Fujii N, Ushida T, Tateishi T. Osteopontin involvement in integrin-mediated cell signaling and regulation of expression of alkaline phosphatase during early differentiation of UMR cells. FEBS Lett 1997; 420:112-116.
33. Mizuno M, Fujisawa R, Kuboki Y. Type I collagen-induced osteoblastic differentiation of bone-marrow cells mediated by collagen-$\alpha_2\beta_1$ integrin interaction. J Cell Physiol 2000; 184:207-213.
34. Altankov G, Grinnell F, Groth T. Studies on the biocompatibility of materials: fibroblast reorganization of substratum-bound fibronectin on surfaces varying in wettability. J Biomed Mater Res 1996; 30:385-391.
35. Rupp F, Scheideler L, Rehbein D, Axmann D, Geis-Gerstorfer J. Roughness-induced dynamic changes of wettability of acid etched titanium implant modifications. Biomaterials 2004; 25:1429-1438.
36. McBeath R, Pirone DM, Nelson CM, Bhadriraju K, Chen CS. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. Dev Cell 2004; 6:483-495.
37. Alford AI, Jacobs CR, Donahue HJ. Oscillating fluid flow regulates gap junction communication in osteocytic MLO-Y4 cells by an ERK1/2 MAP kinase-dependent mechanism. Bone 2003; 33:64-70.