Attachment of Osteoblasts on Absorbable Adhesive Composite Bone Cements/Fillers

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Statement of Purpose: Recent research efforts in this laboratory on absorbable, adhesive, composite bone cements/fillers have focused on (1) the basic properties of phosphate/cyanoacrylate composites,[1] (2) the effect of reinforcing the phosphate/cyanoacrylate composites with knitted meshes on their properties,[2] and (3) the effect of incorporating $CaSiO_3$ on the basic properties of phosphate/cyanoacrylate composites.[3] Collective analysis of the results of these studies led to the hypotheses that the family of absorbable adhesive bone cements/fillers would (1) be easy to apply and adhere to natural tissue, (2) form a strong bone filler, and (3) facilitate bone regeneration. This provided incentive to pursue the present study, which investigates the effect of surface composition on osteoblast adhesion.

Methods: Bone cements/fillers ABC-1, ABC-4, and ABC-5 were prepared using equal amounts of liquid methoxypropyl cyanoacrylate and a solid inorganic powder as previously described.[3] The inorganic powder consisted of 100% DBCP, 5% + 25% DBCP + $CaSiO_3$, and 45% + 5% DBCP + $CaSiO_3$ in ABC-1, -4, and -5, respectively. To determine the effect of partial degradation on the surface properties, the composites were incubated at 50°C in deionized water for one month. Bone cement pieces (~1 cm^2) were dipped in isopropanol and exposed to UV light in a laminar flow hood. After drying, samples were fixed to the bottom of each well of a 24-well culture plate with sterile vacuum grease. Osteoblast cells (7F2, passage 3-5, ATCC#CRL-12557) were maintained under standard conditions (that is, a humidified, 37ºC, 5% CO_2/95% air environment) until confluent. The cells were then seeded (30,000 to 50,000 cells/cm^2) on the prepared bone filler samples or controls, in triplicate. Controls were seeded directly in the bottom of the tissue-culture treated, polystyrene well. Samples were then incubated at 37°C under standard culture conditions for 4 hours. At the conclusion of this period, the media were aspirated, cells were rinsed with PBS, and either stained for fluorescence or assayed with a cell viability assay (Promega) according to manufacturer instructions. For fluorescence staining, propidium iodide solution [5mg/mL] was added and allowed to incubate at room temperature for 15 minutes. Cells were rinsed with PBS and samples were viewed using fluorescence microscopy.

Results: Osteoblast adhesion on the intact composite surfaces and controls are illustrated by Figures 1-a to 1-d. Qualitatively, the cell attachment decreased progressively from ABC-1 to the control to ABC-5 to ABC-4. Tentatively, this suggests that the silicate-free ABC-1 does encourage cell attachment and the addition of a small fraction of silicate (ABC-4) may counteract this behavior. Upon repeating the cell attachment experiment using partially degraded ABC-1, ABC-4, and ABC-5, the data in Table I show clearly that the number of cells that adhered to the three ABC substrates are significantly lower than the polystyrene control, irrespective of the composition of the inorganic component. This may be related to the loss of $CaSiO_3$ from ABC-4 and ABC-5 and possibly the generation of localized negative charge in the prevailing phosphate component which may discourage cell attachment.

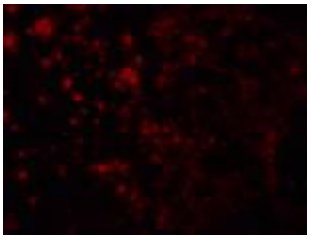

Figure 1-a: Polystyrene Control

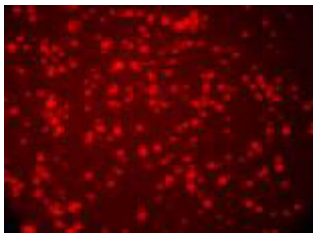

Figure 1-b: ABC-1 Surface

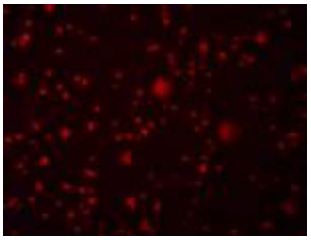

Figure 1-c: ABC-4

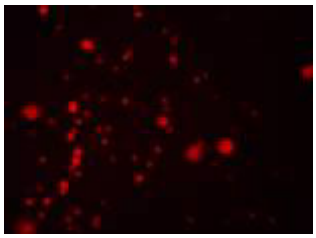

Figure 1-d: ABC-5

Figures 1a-1d: Cell attachment to polystyrene control and intact surfaces of absorbable adhesive composite bone fillers ABC-1, -4, and -5.

Table I. Osteoblast Adhesion onto Partially Degraded, Absorbable Adhesive Composite Bone Fillers

Sample	Composition	Average Cell Adhesion (normalized to control)
Polystyrene Control	Tissue Culture Polystyrene	100%
ABC-5	5% $CaSiO_3$	64%
ABC-4	25% $CaSiO_3$	63%
ABC-1	DBCP*	62%

*Dibasic calcium phosphate

Conclusions: Preliminary results indicate that (1) ABC-1 initially enhances osteoblast attachment, and (2) incorporation of $CaSiO_3$ initially discourages cell attachment.

References:

[1]Shalaby, S.W. et al., Absorbable Self-setting Composite Adhesive Bone Cement/Filler, *Trans. Soc. Biomater.*, (2009) submitted.
[2]Vaughn, M.A. et al., Fiber-reinforced Absorbable, Self-setting, Composite Bone Filler: A Preliminary Report, *Trans. Soc. Biomater.*, (2009) submitted.
[3]Nagatomi, S.D. et al., Effect of Incorporating $CaSiO_3$ on the Properties of Absorbable, Adhesive Composite Bone Cement/Filler, *Trans. Soc. Biomater.*, (2009) submitted.