

# Pengaruh Ukuran Granul Prototipe Karbonat Hidroksiapatit terhadap Kelarutan In Vitro dan Sitotoksitas = Effect of Carbonate Hydroxyapatite Prototype Granule Size on In Vitro Solubility and Cytotoxicity

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## Abstrak

Latar Belakang: Perbaikan defek tulang dapat dilakukan dengan penggunaan material bone graft. Secara klinis, material bone graft dipakai dalam berbagai variasi ukuran granul yang disesuaikan dengan pertimbangan untuk aplikasi spesifik dan tujuan penggunaan bone graft. Di sisi lain, informasi mengenai pengaruh ukuran granul bone graft belum diketahui dan belum ada penelitian mengenai efek perbedaan ukuran granul pada prototipe karbonat hidroksiapatit terhadap kelarutan in vitro dan sitotoksitas. Tujuan: Mengevaluasi pengaruh ukuran granul terhadap kelarutan in vitro dan sitotoksitas prototipe karbonat hidroksiapatit. Metode: Uji kelarutan in vitro dengan merendam prototipe karbonat hidroksiapatit dalam larutan asetat buffer dan Tris-HCl buffer selama 7 hari dalam suhu 37°C. Nilai kelarutan in vitro diuji dengan alat Horiba Ion Selective Electrode. Uji sitotoksitas dengan MTT Assay pengaruh medium ekstrak terhadap sel preosteoblas MC3T3-E1 selama 1 hari. Nilai absorbansi dibaca dengan ELISA Microplate Reader. Analisis data dengan uji statistik One-Way ANOVA. Hasil: Konsentrasi ion kalsium terlarut dalam larutan asetat buffer pada kelompok ukuran granul 250-500 µm yaitu  $45,79 \pm 3,11$  mg/L, 500-1000 µm yaitu  $37,41 \pm 4,28$  mg/L, dan 1000-2000 µm yaitu  $35,85 \pm 1,28$  mg/L. Konsentrasi ion kalsium terlarut dalam larutan Tris-HCl buffer pada kelompok ukuran granul 250-500 µm yaitu  $3,88 \pm 0,36$  mg/L, 500-1000 µm yaitu  $2,94 \pm 0,19$  mg/L, dan 1000-2000 µm yaitu  $2,02 \pm 0,58$  mg/L. Uji statistik menunjukkan perbedaan konsentrasi ion kalsium terlarut yang signifikan antara granul ukuran 250-500 µm dan 1000-2000 µm pada kedua larutan buffer. Persen viabilitas sel menunjukkan hasil diatas 70% pada semua kelompok ukuran granul dan konsentrasi ekstrak. Uji statistik menunjukkan perbedaan nilai absorbansi dan persen viabilitas sel pada konsentrasi ekstrak 50 mg/mL prototipe karbonat hidroksiapatit ukuran 250-500 µm terhadap 1000-2000 µm, 100 mg/mL prototipe karbonat hidroksiapatit ukuran 250-500 µm terhadap 500-1000 µm, dan konsentrasi ekstrak 200 mg/mL prototipe karbonat hidroksiapatit ukuran 250-500 µm terhadap 1000-2000 µm dan 500-1000 µm terhadap 1000-2000 µm. Kesimpulan: Semakin besar ukuran granul prototipe karbonat hidroksiapatit yang direndam dalam larutan asetat buffer dan Tris-HCl buffer, hasil uji kelarutan in vitro menunjukkan rata-rata konsentrasi ion kalsium terlarut yang semakin kecil. Uji sitotoksitas prototipe karbonat hidroksiapatit menunjukkan hasil nontoksik dengan viabilitas sel ukuran granul 1000-2000 µm > 500-1000 µm > 250-500 µm.

.....Background: Repair of bone defects can be done using bone graft material. Clinically, bone graft material is used in a variety of granule sizes that are adjusted to consider the specific application and intended use of the bone graft. On the other hand, information regarding the influence of bone graft granule size is not yet known and there has been no research regarding the effect of differences in granule size in hydroxyapatite carbonate prototypes on in vitro solubility and cytotoxicity. Objective: To evaluate the effect of granule size on the in vitro solubility and cytotoxicity of the carbonate hydroxyapatite prototype. Methods: The in vitro solubility test was conducted by immersing the carbonate hydroxyapatite prototype in

a solution of acetate buffer and Tris-HCl buffer for 7 days at a temperature of 37°C. The in vitro solubility value was tested using the Horiba Ion Selective Electrode. The cell cytotoxicity test was carried out using MTT assay for the effect of medium extracts on MC3T3-E1 preosteoblast cells. Absorption values were read with an ELISA Microplate Reader. Data analysis using the One-Way ANOVA statistical test. Results: The concentration of dissolved calcium ions in the acetate buffer solution in the granule size group 250-500  $\mu\text{m}$  was  $45.79 \pm 3.11$  mg/L, 500-1000  $\mu\text{m}$  was  $37.41 \pm 4.28$  mg/L, and 1000-2000  $\mu\text{m}$  was  $35,85 \pm 1.28$ mg/L. The concentration of dissolved calcium ions in the Tris-HCl buffer solution in the granule size group 250-500  $\mu\text{m}$  was  $3.88 \pm 0,36$  mg/L, 500-1000  $\mu\text{m}$  was  $2.94 \pm 0.19$  mg/L, and 1000-2000  $\mu\text{m}$  was  $2,02 \pm 0.58$ mg/L. Statistical tests showed significant differences in dissolved calcium ion concentrations between granules measuring 250-500  $\mu\text{m}$  and 1000-2000  $\mu\text{m}$  in both buffer solutions. The percentage of cell viability showed results above 70% in all granule size groups and extract concentrations. Statistical tests show differences in absorption values and percent cell viability at extract concentrations of 50 mg/mL of carbonate hydroxyapatite prototype with a size of 250-500  $\mu\text{m}$  versus 1000-2000  $\mu\text{m}$ , 100 mg/mL hydroxyapatite carbonate prototype with a size of 250-500  $\mu\text{m}$  versus 500-1000  $\mu\text{m}$ , and extract concentration 200 mg/mL carbonate hydroxyapatite prototype size 250-500  $\mu\text{m}$  against 1000-2000  $\mu\text{m}$  and 500-1000  $\mu\text{m}$  against 1000-2000  $\mu\text{m}$ . Conclusion: The larger the size of the carbonate hydroxyapatite prototype granules soaked in acetate buffer and Tris-HCl buffer, the results of the in vitro solubility test show a smaller average concentration of dissolved calcium ions. The cytotoxicity test of the carbonate hydroxyapatite prototype showed nontoxic results with cell viability of granule size 1000-2000  $\mu\text{m}$  > 500-1000  $\mu\text{m}$  > 250-500  $\mu\text{m}$ .