

Conclusions and Recommendations

In the treatment of bone defects there are many bone graft procedures performed in order to substitute and restore lost tissue. Injectable, in situ polymerizable, biodegradable scaffold that can guide bone regeneration while providing structural support to the region will alleviate many of the problems encountered with current treatment methods.

Calcium sulfate compounds are becoming of increasingly great importance in the field of biomaterials. Calcium sulfate-based bioceramics have been used successfully in traumatology, dentistry and maxilla–facial surgery for over 40 years for bone repairing, primarily because of their biocompatibility, bioactivity and osteoconductivity. Disadvantages of calcium sulfate are its transient cytotoxic effect leading to inflammatory reactions, it is completely resorbed following implantation and it has no compressive strength *in vivo*. In this study these disadvantages were tolerated by doping CSD with LS water extract (the amount of the dopant is critical).

The FTIR, Raman spectroscopy, DMA, SEM-EDX and GC/MS analysis for bone mineral, CSD, LS with different bone composites were carried. The crystallinity of bone minerals, crystal size, type B carbonate substitution, the variation in the carbonate to phosphate ratio, the acid phosphate content and the dissolving properties together with a comparison of the compressive properties among all the composites under investigation were also tested.

Incorporation of cortical grafts is initiated by osteoclasts rather than osteoblasts. The acidic environment is essential for bone resorption. Bone growth and remodeling are normal physiological events that occur at a high rate throughout childhood and

adolescence, and to a much lesser extent during adult years. It is the net result of the activity of two types of bone cell which have opposing actions: those that synthesis new bone material, mainly, osteoblasts, and cells called osteoclasts, which are responsible for resorbing or breaking down existing bone material.

The increases in acid phosphate content (maximum value was detected in composite (BM+CSD+LS) is considered now as a great advantage of these composites thus, acid phosphate stimulates bone remodeling in a way it stimulates osteoclast to start bone resorption.

The unique balance between matters that stimulate bone resorption and that stimulate bone formation makes the composite (BM+CSD+LS) the optimum composite for use in bone grafting. Since it is now evident that the presence of squaline and Zn ions inhibits bone resorption, stimulate alkaline phosphatase (ALP) activity, and stimulate osteoblast for bone formation leading to acceleration of osteogenesis of osteoblasts.

Zinc ions act to increase bone formation, mineralization and decreases bone resorption, it is also the reason of increasing the compressive strength of BM+CSD+LS composite by 1.5 fold compared to the (BM+CSD) and (BM+LS) composites.

Furthermore, the presence of [sinapic acid has stimulatory effects on bone formation and inhibitory effects on bone resorption, and butanoic acid has stimulates bone sialoprotein BSP gene transcription, (BSP) is thought to function in the initial mineralization of bone, and may be crucial for osteoblast differentiation, and bone matrix mineralization, *Silane* showed excellent osteoconductivity and strong enough mechanical properties for clinical use and squaline was used as an antidote to reduce drug toxicity] in LS water extract and it helped in bone remodeling, increasing the

compressive strength and overcoming of the transit cytotoxic effect of CSD when used as a bone substitute material.

Incorporation of the amino acids, which occurs through a specific interaction with the HA structure, affects the degree of crystallinity of the apatite phase, which decreases as the amino acid content increases. The presence of the acidic amino acids allows controlling the crystal dimensions and favors osteoblasts proliferation, activation of their metabolism and differentiation, which is of high importance for potential biomedical applications.

The crystallinity, i.e. the HA crystal size and perfection of apatites are of great biomedical interest, because smaller, more imperfect crystals, being subject to dissolution, may affect the extent of bone loss for example in osteoporosis and other metabolic bone disease. Thus, doping (BM+CSD) with LS water extract increases the dissolving time which may help if tested *in vivo* to limit the fast resorbable rate of CSD and controlling both the crystallinity and the crystal size of bone apatite.

Carbonation of mineral tends to increase in normal bone as the mineral matures. The (BM+CSD) composite only leads to an increase in type B carbonate substitution with also an increase in amorphous phosphate due to the transfer part of CSD to form calcium phosphate deposits, in the presence of water, while in LS composites another substitution takes place.

Doping CSD with the LS water extract limits the degree of type B-carbonate substitution. To conclude addition of CSD to bone mineral leads to a decrease in crystallinity while adding LS only or doping CSD with LS increases the crystallinity of bone mineral apatite compared to (BM+CSD) composite along with increasing in the apatite crystal size. Doping CSD with LS leads also to a valuable increase in the compressive strength of composite (BM+CSD+LS) compared to (B+CSD) or

(B+LS) composites which proves another types of bone substitution rather than type B carbonate substitution and also increasing the dissolving time.

If tested *in vivo* the (BM+CSD+LS) composite would be a very promising bone substitute materials, which could be osteogenesis, has a remarkable compressive strength, possess anti-inflammatory, antifungal and antibacterial effects and can remain for a longer time in the defected bone.

Sabinene contains anti-inflammatory, antifungal and antibacterial effect to the composition of calcium sulfate which alters the physical and morphological properties of the composite and improves its compressive strength, the presence of synaptic acid, *silane*, sabinene, and aspartic acid. and ions such as Ca^{+2} , Zn^{+2} having stimulatory effects on bone formation into calcium sulfate implants which makes the composite (BM+CSD+LS) the optimum composite that can be used in bone grafting.

List of References

List of References

- Adele, L. and Richard, M. (2005) Infrared spectroscopic characterization of mineralized tissues, Vib Spectrosc, vol. 38: 107–114.
- Ahsan, S. K., Tariq, M., Ageel, M., Al-Yahya, M. A. and Shah, A., H. (1989) Studies on some herbal drugs used in fracture healing, Crude Drug Res, vol. 27: 235–239.
- Alexander, D. I., Manson, N. A. and Mitchell, M. J. (2001) Efficacy of calcium sulfate plus decompression bone in lumbar and lumbosacral spinal fusion: Preliminary results in 40 patients, Can J Surgery, vol. 44: 262–266.
- Alexander, J. W. (1987) Bone grafting, Clin North Am Small Anim Pract vol. 811–819.
- Anthony, A., Beardmore, M. D., Daniel, E., Brooks, B., Joseph, C., Wenke, P., Darryl, B. and Thomas, M. D. (2005) Effectiveness of local antibiotic delivery with an osteoinductive and osteoconductive bone-graft substitute, The Journal of Bone & Joint Surgery, vol. 87: 107-112
- Archana, N. P. and Anita, A. M. (1996) A study on clinical efficacy of lepidium sativum seeds in treatment of bronchial asthma, Pharmacol. Ther, vol. 5: 55-59.
- Aspenberg, P. and Turek, T. (1996) Bmp-2 for intramuscular bone induction: Effect in squirrel monkeys is dependent on implantation site, Acta Orthop Scand, vol. 67: 3-6.
- Athanasίου, K. A., Zhu, C. F., Lanctot, D. R., Agrawal, C. M. and Wang, X. (2000) Fundamentals of biomechanics in tissue engineering of bone, Tissue Engineering, vol. 6: 361-381.
- Atkins, R. C. (2009) Squalene: Oxygenator, cancer fighter, Edited by: Simon and Schuster, UK.
- Auer, J. (1992) Principles in fracture treatment, Edited by : Saunders, Philadelphia.
- Bailey, R. J. and Holt, C. (1988) Fourier transform infrared spectroscopy and characterization of biological calcium phosphates, Edited by: Hukins, D. W. L. Boca Raton: CRC Press Inc.
- Bajpai, P. K. (1992) A novel device for sustained long term delivery of drugs, Bioceramics, vol. 3: 87–89.
- Bart, C. (2008) Normal bone anatomy and physiology, Clin J Am Soc Nephrol, vol. 3: S131–S139.
- Baser, K. H. C. and Buchbauer, G. (2010) Essential oils: Science, technology, and applications, Edited by: Eskişehir, Boca Raton : CRC Press NW.
- Bassett, C. (1972) Clinical implications of cell function in bone grafting, Clin Orthop Rel Res, vol. 87: 49.
- Beavis, R. C. and Chait, B. T. (1989) Matrix-assisted laser-desorption mass spectrometry using 355 nm radiation, Rapid Commun. Mass Spectrom, vol. 3: 436–439.
- Bell, W. H. (1964) Resorption rates of bone and bone substitutes, Oral Surg, vol. 17: 650–657.
- Bettger, W. J. and Dell, B. L. (1993) Physiological roles of zinc in the plasma membrane of mammalian cells, Nutr Biochem, vol. 4: 194–207.

- Bohner, M. (2000) Calcium orthophosphates in medicine: From ceramics to calcium phosphate cements, Injury, vol. 31: 37-47.
- Bonel, G. (1972b) Contribution a l'etude de la carbonation des apatites. Parts ii and iii, Ann. Chim, vol. 7: 127-144.
- Bostrom, M., Lane, J. M. and Tomin, E. (1996) Use of bone morphogenetic protein-2 in the rabbit ulnar nonunion model, Clin Orthop, vol. 272-282.
- Boyne, P. J. (1996) Animal studies of application of rhbmp-2 in maxillofacial reconstruction, Bone, vol. 83S-92S.
- Bryan, R. M., Shailesh, N. S., Jill, K. W., F.V., S. and Roque, L. E. (2009) Composition and physical properties of cress (*lepidium sativum* l.) and field pennycress (*thlaspi arvense* l.) oils, Indus. Crops Prod, vol. 30: 199-205.
- Carden, A. and Morris, M. D. (2000) Application of vibrational spectroscopy to the study of mineralized tissues, J Biomed Opt, vol. 5: 259-268.
- Carden, A., Rajachar, R. M., Morris, M. D. and Kohn, D. H. (2003) Ultrastructural changes accompanying the mechanical deformation of bone tissue: A raman imaging study, Calcif Tissue Int, vol. 72: 166-175.
- Carinci, F., Piattelli, A., Stabellini, G., Palmieri, A., Scapoli, L., Laino, G., Caputi, S. and Pezzetti, F. (2004) Calcium sulfate: Analysis of mg63 osteoblast-like cell response by means of a microarray technology, Biomedical Materials Research vol. 71B: 260.
- Chipchase, L., Mccaul, K. and Hearn, T (2000) Hip fracture rates in South Australia: Into the next century, Australian and New Zealand, Surgery, vol. 70: 117-119.
- Coen, G., Ballanti, P. and Bonucci, E. (1998) Bone markers in the diagnosis of low turnover osteodystrophy in haemodialysis patients, Nephrol Dial Transplant vol. 13: 2294-2302.
- Coetzee, A. S. (1980) Regeneration of bone in the presence of calcium sulphate, Arch. Otolaryngol., vol. 106: 405-409.
- Colletti, L. A. J., Edwards, L., Gordon, J., Shary, c. and Bell, N. H. (1989) The effects of muscle-building exercise on bone mineral density of the radius, spine, and hip in young men, Calcif Tissue, vol. 4: 12-14.
- Conforti, F., Sosa, S., Marrelli, M., Menichini, F., Statti, G. A., Uzunov, D., Tubaro, A. and Menichini, F. (2009) The protective ability of mediterranean dietary plants against the oxidative damage: The role of radical oxygen species in inflammation and the polyphenol, flavonoid and sterol contents, Food Chemistry, vol. 112: 587-594.
- Constantino, P. D. and Freidman, C. D. (1994) Synthetic bone graft substitutes, Otolaryngol. Clin. North Am., vol. 27: 1037-1073.
- Crupi, V., Majolino, D., Migliardo, P., Mondello, M. R., German, M. P. and Pergolizzi, S. (2001) Ftir molecular evidence of liver damage by chemical agents, Vibrational Spectroscopy, vol. 25: 213-222.
- Currey, J. D. (1998) Mechanical properties of vertebrate hard tissues, Mechanical Engineers, vol. 212: 399-411.
- Cypher, T. J. and Grossman, J. P. (1996) Biological principles of bone graft healing, Foot Ankle Surg, vol. 35: 413-417.
- Czimer, G. and Szabo, L. (1988) Therapeutical effect and production of garden cress (*Lepidium Sativum* L), Gyogyszereszet, vol. 32: 79-81.
- Dacquet, V., Varlet, A., Tandogan, R. N., Tahon, M. M., Fournier, L., Jehl, F., Monteil, H. and Bascoulergue, G. (1992) Antibiotic impregnated plaster of paris beads trials with teicoplanin, Clin Orthop vol. 282: 241-249.

- Damien, C. J. and Parsons, J. R. (1991) Bone grafts and bone graft substitutes: A review of current technology and applications, Appl Biomater, vol. 2: 187–208.
- De Groot, K. (1983) Ceramic of calcium phosphate: Preparation and properties, Edited by: Boca, R, CRC Press Inc.
- De Groot, K. (1993) Clinical applications of calcium phosphate biomaterials: A review, Ceramics International vol. 19: 363-366.
- De Long, W. G., Einhorn, T. A., Koval, K. C., M.F. M., Smith, W. O. and Sanders, R. (2007) Bone grafts and bone graft substitutes in orthopedic trauma surgery – a critical analysis, J Bone Joint Surg Am, vol. 89A: 649–658.
- Delacure, M. D. (1994) Physiology of bone healing and bone grafts, Otolaryngol Clin North Am, vol. 27: 859-873.
- Diwakara, B. T., Duttaa, P. K., Lokeshb, B. R. and Naidu, K. A. (2008) Bioavailability and metabolism of n-3fatty acid rich garden cress (*lepidium sativum*) seed oil in albino rats, Prostaglandins, Leukotrienes and Essential Fatty Acids, vol. 78: 123-130.
- Doadrio, J. C., Arcos, D., Cabanas, M. V. and Vallet-Regi, M. (2004) Calcium sulphate-based cements containing cephalixin, Biomaterials, vol. 25: 2629.
- Donnellya, E., Rebecca, M., Williamsa, S. A., Downsa, A., Michelle, E., Dickinsona, A., Shefford, P., Bakera, B. and Marjolein, C. H. (2006) Quasistatic and dynamic nanomechanical properties of cancellous bone tissue relate to collagen content and organization, Journal of Materials Research, vol. 21: 2106-2117.
- Ducheyne, P. and Qiu, Q. (1999) Bioactive ceramics: The effect of surface reactivity on bone formation and bone cell function, Biomaterials, vol. 2287-2303.
- Eddouks, M., Maghrani, M., Zeggwagh, N. A. and Michel, J. B. (2005) Study of the hypoglycaemic activity of *lepidium sativum* l. Aqueous extract in normal and diabetic rats, J. Ethano. Pharmacol, vol. 97: 391–395.
- Elguera, J. C., Barrientos, E. Y., Wrobel, K. and Wrobel, K. (2013) Effect of cadmium (Cd(II)), selenium (Se(IV)) and their mixtures on phenolic compounds and antioxidant capacity in *Lepidium sativum*, Acta Physiologiae Plantarum vol. 35: 431 - 441.
- Elisa, B., Paola, T., Massimo, G., Roberto, G. and Adriana, B. (2006) Nanocomposites of hydroxyapatite with aspartic acid and glutamic acid and their interaction with osteoblast-like cells, Chair of Surgical Pathophysiology, vol. 25: 4428-4433
- Elliott, J. C. (1962) The infrared spectrum of the carbonate ion in carbonate-containing apatites, Dent Res, vol. 30: 1284.
- Emerson, W. H. and Fischer, E. E. (1962) The infra-red absorption spectra of carbonate in calcified tissues, Arch Oral Biol, vol. 7: 671-683.
- Fahey, J. W., Zalcman, A. T. and Talalay, P. (2001) The chemical diversity and distribution of glucosinolates and isothiocyanates among plants, Phytochemistry, vol. 56: 5-51.
- Franceschi, R. T. and Iyer, B. S. (1992) Relationship between collagen synthesis and expression of the osteoblast phenotype in mc3t3-e1 cells, Bone Miner Res, vol. 7: 235–246.
- Freeman, M. J. and Silva, M. J. (2002) Separation of the raman spectral signatures of bioapatite and collagen in compact mouse bone bleached with hydrogen peroxide, Applied Spectroscopy, vol. 56: 770-775.

- Giovanna, G., Alfredo, D., Paola, L. and Mario, M. (2007) Development of a new calcium sulphate-based composite using alginate and chemically modified chitosan for bone regeneration, Wiley Inter Science, vol. 34: 811-20
- Gitelis, S. and Grebach, G. T. (2002) The treatment of chronic osteomyelitis with a biodegradable antibiotic impregnated implant, J Orthop Surg Clin North Am, vol. 10: 53–60.
- Gokavi, S. S., Malleshi, N. G. and Guo, M. (2004) Chemical composition of garden cress (*lepidium sativum*) seeds and its fractions and use of bran as a functional ingredient, plant food, Hum. Nutr, vol. 59: 105–111.
- Goldburg, V. (1989) Bone Transplantation, surgery of the musculoskeletal system, Edited by: Bucholz and Mooney, New York: Evarts C
- Goren, S., Gokbayrak, H. and Altıntaş, S. (2004). Production of Hydroxylapatite From Animal Bone, Engineering Materials, vol. 264-268: 1949-1952.
- Greenwald, A. S., Boden, S. D., Goldberg, V. M., Khan, Y., Laurencin, C. T. and Rosier, R. N. (2001) The committee on biological implants. Bone-graft substitutes: Facts, fictions, and applications, J. Bone Joint Surg Am., vol. 83: 98-103.
- Griffith, L. G. and Naughton, G. (2002) Tissue engineering – current challenges and expanding opportunities, Science, vol. 295: 1009-1020.
- Gunasekaran, K. and Thotapallip, S. (2011) Preparation and characterization of a novel bone graft composite containing bone ash and egg shell powder, Indian Academy of Sciences, vol. 34: 177-181.
- Guyton, C. (1991) Textbook of medical physiology, Edited by: W.P Saunders,
- Hak, D. J. (2007) The use of osteoconductive bone graft substitutes in orthopaedic trauma, J Am Acad Orthop Surg vol. 15: 525-536.
- Hashizume, M. and Yamaguchi, M. (1993) Stimulatory effect of beta-alanyl-L-histidinato zinc on cell proliferation is dependent on protein synthesis in osteoblastic mc3t3-e1 cells, Mol Cell Biochem, vol. 122: 59–64.
- Hashizume, M. and Yamaguchi, M. (1994) Effect of beta-alanyl-L-histidinato zinc on differentiation of osteoblastic mc3t3-e1 cells: Increases in alkaline phosphatase activity and protein concentration, Mol Cell Biochem, vol. 131: 19–24.
- Hesaraki, S., Nemati, R. and Nazarian, H. (2009) Physico-chemical and in vitro biological study of zinc-doped calcium sulfate bone substitute, J Biomed Mater Res B Appl Biomater, vol. 91: 37-45.
- Hollinger, J. O. and Battistone, G. C. (1986) Biodegradable bone repair materials, Clin. Orthop., vol. 207: 290–305.
- Hollinger, J. O. and Seyfer, A. E. (1994) Bioactive factors and biosynthetic materials in bone grafting, Clin Plast Surg, vol. 21: 415-418.
- Hou, L. T., Liu, C. M., Liu, B. Y., Chang, P. C., Chen, M. H. and Ho, M. H. (2007) Tissue engineering bone formation in novel recombinant human bone morphogenic protein 2-atelocollagen composite scaffolds, Periodontol, vol. 78: 335–343.
- Hulth, A. (1989) Current concepts of fracture healing, Clinical Orthopaedics and Related Research, vol. 249: 265-284.
- Ilan, D. I. and Ladd, A. L. (2003) Bone graft substitutes, Operat Tech Plast Reconstr Surg, vol. 9: 151–160.
- Isaksson, H., Malkiewicz, M., Nowak, R., Helminen, H. J. and Jurvelin, J. S. (2010) Rabbit cortical bone tissue increases its elastic stiffness but becomes less viscoelastic with age, Bone, vol. 47: 1030-1038.

- James, D. K., Simon, Y., Leda, K., Mark, W. and Antonios, G. M. (2009) Injectable biomaterials for regenerating complex craniofacial tissues, Adv Mater, vol. 21: 3368–3393.
- Jee, W. S. S. (1983) The skeletal tissues, Edited by: Weiss, L.
- Jerome, G. S. and Debi, P. M. (2009) In vivo testing of a bone graft containing chitosan, calcium sulfate and osteoblasts in a paste form in a critical size defect model in rats, Biomedical Science and Engineering, vol. 2: 24-29.
- Jitomir, J. and Willoughby, D. S. (2008) Leucine for retention of lean mass on a hypocaloric diet, Leucine for retention of lean mass on a hypocaloric diet, vol. 11: 606-609.
- Johnson, A. L. (1991) Principles of bone grafting., Semin Vet Med Surg, vol. 6: 90-99.
- Kamimura, H., Koga, N., K., O., Yoshimura, H., Inoue, H., Sato, K. and Ohkubo, M. (1989) Studies on distribution, excretion and subacute toxicity of squalene in dogs., Fukuoka Igaku Zasshi, vol. 80: 269-280.
- Kassie, F., Laky, B., Gminski, R., Lhoste, E. and Kansmuller, S. (2003) Effects of garden and water cress juices and their constituents, benzyl and phenethyl isothiocyanates, towards benzo(a)pyrene-induced DNA damage: A model study with the single cell gel electrophoresis/hep g2 assay, Chemical Biology Interactions, vol. 3: 285–296.
- Kelly, C. M., Wilkins, R. M., Gitelis, S., Hartjen, C., Watson, J. T. and Kim, P. T. (2001) The use of a surgical grade calcium sulfate as a bone graft substitute: Results of a multicenter trial, Clin Orthop, vol. 382: 42-50.
- Kishi, S. and Yamaguchi, M. (1994) Inhibitory effect of zinc compounds on osteoclast-like cell formation in mouse marrow culture, Biochem Pharmacol, vol. 48: 1225–1230.
- Klokkevold, P. R. and Jovanovic, S. A. (2002) Advanced implant surgery and bone grafting techniques, Edited by: Takei and Carranza, Philadelphia: W. B. Saunders.
- Kold, S. E. and Hickman, J. (1983) Use of an autogenous cancellous bone graft in the treatment of subchondral bone cysts in the medial femoral condyle of the horse., Equine Vet, vol. 15: 312-316.
- Komath, M. and Varma, H. K. (2003) Development of a full injectable calcium phosphate cement for orthopedic and dental applications, Bull Mater Sci, vol. 26: 415–422.
- Komath, M., Varma, H. K. and Sivakumar, R. (2000) On the development of an apatitic calcium phosphate bone cement, Bull. Mater. Sci, vol. 23: 135–140.
- Kotha, S. P., Lieberman, M., Vickers, A., Schmid, S. R. and Mason, J. J. (2006) Adhesion enhancement of steel fibers to acrylic bone cement through a silane coupling agent, J Biomed Mater Res A, vol. 76: 111-119.
- Lai, Y. L. and Yamaguchi, M. (2009) Phytocomponent p-hydroxycinnamic acid stimulates bone formation and inhibits bone resorption in rat femoral tissues in vitro., Mol Cell Biochem, vol. 292: 45-52.
- Laurencin, C. A., Khan, Y. M. and El-Amin, S. F. (2006) Bone graft substitutes, Expert Rev Med Devices vol. 3: 49–57.
- Layman, D. K. (2003) The role of leucine in weight loss diets and glucose homeostasis^{1,2}, Society for Nutritional Sciences, vol. 133: 261S-267S
- Legeros, R. Z. (1981) Apatites in biological systems, Prog. Crystal. Growth Charact., vol. 4: 1-45.

- Linde, A. and Hedner, E. (1995) Recombinant bone morphogenetic protein-2 enhances bone healing, guided by osteopromotive e-PTFE membranes: an experimental study in rats, Calcif Tissue Int, vol. 56: 549-553.
- Lodish, H. (1995) Molecular cell biology Edited by: Sakakibara, H. New York: Scientific American Books, Natl. Acad.
- Lu, S., Zhang, Z., and Wang, J. (2004) Guided bone regeneration in long bone, Chin Med, vol. 551-554.
- Maghrani, M., Zeggwagh, N. A., Michel, J. B. and Eddouks, M. (2005) Antihypertensive effect of lepidium sativum l. In spontaneously hypertensive rats, Journal of Ethnopharmacology, vol. 100: 193-197.
- Maier, U. H., Gundlach, H. and Zenk, M. H. (1998) Seven imidazole alkaloids from lepidium sativum, Phytochemistry, vol. 49: 1791-1795.
- Mao, D., Lookman, R., Weghe, H. V., Weltens, R., Vanermen, G. and Brucker, N. (2009) Combining HPLC-GC/MS, GC/MS/ToF-MS, and selected ecotoxicity assays for detailed monitoring of petroleum hydrocarbon degradation in soil and leaching water, Environ Sci Technol, vol. 15: 7651-7.
- Marra, K. G., Szem, J. W., Kumta, P. N., Dimilla, P. A. and Weiss, L. E. (1999) In vitro analysis of biodegradable polymer blend/hydroxyapatite composites for bone tissue engineering, Biomed Mater Res, vol. 47: 324-335.
- Mckee, M., Wild, L., Schemitsch, E. and Waddell, J. (2002) The use of an antibiotic-impregnated, osteoconductive, bioabsorbable bone substitute in the treatment of infected long bone defects: Early results of a prospective trial, J Orthop Trauma, vol. 16: 622-627.
- Meekeren, J. V. (1668) Heel-en Geneeskunstige Aanmerkingen, Commelijn, vol. 82: 539-546.
- Mirza, M. and Navaei, M. N. (2006) Essential oil composition of lepidium sativum, Iranian Journal of Medicinal and Aromatic Plants, vol. 21: 481-488.
- Mirzayan, R., Panossian, V., Avedian, R., Forrester, D. M. and Menendez, L. R. (2001) The use of calcium sulfate in the treatment of benign bone lesions, J Bone Jt Surg, vol. 83-A: 355-358.
- Mohamed, Z., Knözinger, H., Tesche, B. and Mekhemer, G. A. (2006) Influence of phosphonation and phosphation on surface acid-base and morphological properties of cao as investigated by in situ FTIR spectroscopy and electron microscopy, J Colloid Interface Sci, vol. 303: 9-17.
- Moonga, B. S. and Dempster, D. W. (1995) Zinc is a potential inhibitor of osteoclastic bone resorption in vitro, Bone Miner Res, vol. 10: 453-457.
- Murashima, Y., Yoshikawa, G., Wadachi, R., Sawada, N. and Suda, H. (2002) Calcium sulphate as a bone substitute for various osseous defects in conjunction with apicectomy, Int. Endod, vol. 35: 768.
- Nadkarni, S. K. M. (1995) A focus of tissue necrosis increase of renal, Indian medica medica, vol. 1: 736.
- Nancy, P., Adele, B. and Richard, M. (1991) Novel infrared spectroscopic method for the determination of crystallinity of hydroxyapatite minerals, Biophysical Society, vol. 786-793.
- Nather, A., Ong, H. J. C. and Aziz, Z. (2005) Structure of bone, Edited by: World Scientific Publishing Co. Pte. Ltd.
- Navarro, M., del Valle, S., Martinez, S., Zeppetelli, S., Ambrosio, L., Planell, J.A. and Ginebra, M.P. (2004). New macroporous calcium phosphate glass ceramic for guided bone regeneration, Biomaterials, vol. 25: 4233-4241.

- Nikolaos, K. and Margaret, T. (2010) Spectroscopic assessment of normal cortical bone: Differences in relation to bone site and sex, Scientific World, vol. 10: 402–412.
- Nyquist, R. A., Putzig, C. L. and Leugers, M. A. (1997) The handbook of infrared and raman spectra of inorganic compounds and organic salts Edited by: San Diego, USA: Academic Press.
- Nystrom, G. (1928) Plugging of bone cavities with rivanol-plaster porridge, Acta Chir Scand, vol. 63: 296.
- Ong, J. L. and Chan, D. C. N. (1999) Hydroxyapatite and their use as coatings in dental implants: A review, Biomedical Engineering, vol. 28: 667-707.
- Orsini, G., Ricci, J., Scarano, A., Pecora, G., Petrone, G., Iezzi, G. and Piattelli, A. (2004) Bone-defect healing with calcium-sulfate particles and cement: An experimental study in rabbit, J Biomed Mater Res B Appl Biomater, vol. 68: 199-208.
- Ou-Yang, E. P., Paschalis, W. E., Mayo, A. L., Boskey, A. and Mendelsohn, R. (2001) Infrared microscopic imaging of bone: Spatial distribution of CO_3^{2-} , Bone and Mineral Research, vol. 16: 893-900
- Parikh, S. N. (2002) Bone graft substitutes in modern orthopedics, Orthopedics vol. 1301–9.
- Paschalis, E. P., Betts, F., Mendelsohn, R. and Boskey, A. L. (1996) Fourier transform infrared spectroscopy of the solution-mediated conversion of amorphous calcium phosphate to hydroxyapatite: New correlations between x-ray diffraction and infrared data, Calcif. Tissue, vol. 58: 9–16.
- Paschalis, E. P., Burr, D. B., Mendelsohn, R., Hock, J. M. and Boskey, A. L. (2003) Bone mineral and collagen quality in humeri of ovariectomized cynomolgus monkeys given rhPTH(1-34) for 18 months, J Bone Miner Res, vol. 18: 769-775
- Pecora, G., Andreana, S., Margarone, J. E., Covani, U. and Sottosanti, J. S. (1997) Bone regeneration with a calcium sulfate barrier, Oral Surg. Oral Med. Oral Pathol Oral Radiol Endod, vol. 48: 424.
- Pecora, G., De Leonardis, D. and Ricci, J. (2001) L'uso del solfato di calcio nelle tecniche rigenerative multidisciplinari, Edizioni Elite Service, vol. 48 13–77.
- Peltier, L. F. (1961) The use of plaster of paris to fill defects in bone, Clin Orthop, vol. 21: 1-29.
- Peltier, L. F. and Bickel, E. Y. (1957) The use of plaster of paris to fill defects in bone, Ann. Surg., vol. 146: 61-80.
- Petrova, A. (1928) Gipsfüllung von Knochenhöhlen bei Osteomyelitis, Zentralorg Ges Chir, vol. 43: 485.
- Petrova, A. (1928) Gipsfüllung von Knochenhöhlen bei osteomyelitis, Zentralorg Ges Chir vol. 43: 485.
Philadelphia: Academic press.
- Pleshko, N., Boskey, A. and Mendelsohn, R. (1991) Novel infrared spectroscopic method for the determination of crystallinity of hydroxyapatite minerals, Biophys, vol. 60: 786–793.
- Plueddemahn, P. (1991) Silane coupling agents, Edited by: Edwin, V. E, London: Academic Press.
- Polly, B. J. (2008) Transmenopausal changes in the intrinsic material properties of
- Raisz, L. G. (1992) Mechanisms and regulation of bone resorption of osteoclastic cells. In Disorders of bone and mineral metabolism, Edited by: Coe, F. L. and Favus, M. J. New York, Raven Press Ltd.

- Rajesh, R. and Lakshmanan, P. T. (2009) Antioxidant defense of dietary squalene supplementation on sodium arsenite induced oxidative stress in rat myocardium Int. J. Biomed. Pharm. Sci., vol. 2: 98-108.
- Rautaray, D., Mandal, S. and Sastry, M. (2005) Synthesis of hydroxyapatite crystals using amino acid-capped gold nanoparticles as a scaffold, Journal Of Surfaces and Colloids vol. 21: 5185–5191.
- Rehman, A. and Bonfield, M. (1995) Bioceramics, Elsevier Science, vol. 8: 163.
- Rehman, I., Hench, L. L., Bonfield, W. and Smith, R. (1994) Analysis of surface layers on bioactive glasses, Biomaterials, vol. 15: 865-119.
- Rey, C., Collins, B., Goehl, T., Dickson, I. R. and Glimcher, M. J. (1989) The carbonate environment in bone mineral: A resolution-enhanced fourier transform infrared spectroscopy study, Calcif Tissue Int, vol. 45: 157–164.
- Rey, C., Renugopalakrishnan, V., Collins, B. and Glimcher, M. J. (1991) A resolution-enhanced fourier transform infrared spectroscopic study of the environment of the CO_3^{2-} ion in the mineral phase of enamel bone during its formation and maturation, Calcif Tissue Int, vol. 49: 259–268.
- Ricci, J. L., Alexander, H., Nadkarni, P., Hawkins, M., Turner, J., Rosenblum, S., Brezenoff, L., De Leonardis, D. and Pecora, G. (2001) Biological mechanisms of calcium sulfate replacement by bone, Edited by: Davis, J. E, Toronto, Canada: Bone engineering.
- Robert, M. U., Thomas, M. T., Deborah, J. H., Nozomu, I. and Steven, G. (2007) Increased bone formation using calcium sulfate-calcium phosphate composite graft, Clin Orthop Relat Res vol. 459: 110-117.
- Robinson, D., Alk, D., Sandbank, J., Farber, R. and Halperin, N. (1999) Inflammatory reactions associated with a calcium sulfate bone substitute, Ann Transpl vol. 4: 91–97.
- Rumpel, E., Wolf, E., Kauschke, E., Bienengräber, V., Bayerlein, T., Gedrange, T. and Proff, P. (2006) The biodegradation of hydroxyapatite bone graft substitutes in vivo, Folia Morphol (Warsz), vol. 65: 43-48.
- Sadler, p. (1977) Trace elements in human and animal nutrition, Edited by: Underwood, E. J, London: Academic Press.
- Saeed, H., Roghayeh, N. and Hamid, N. (2009) Physico-chemical and in vitro biological study of zinc-doped calcium sulfate, Wiley InterScience vol.
- Sahar, N. D., Hong, S. I. and Kohn, D. H. (2005) Micro- and nano-structural analyses of damage in bone, micron, Bone structure in health and disease, vol. 36: 617-629.
- Sandar, B. G. L., SVETOMIR, Z. M., SUZANA, I., DIMITRIJEVI, A. M. and DEJAN, U. S. (2007) Antimicrobial activity of the essential oil and different fractions of juniperus communis l. and a comparison with some commercial antibiotics, Serbian Chemical Society, vol. 72: 311-320.
- Sharir, A. S., Barak, M. M. and Shahar, R. M. (2008) Whole bone mechanics and mechanical testing, The Veterinary Journal, vol. 117: 8-17.
- Shukla, A., Singh, C. S. and Bigoniya, P. (2011) Phytochemical and CNS activity of lepidium sativum linn. Seeds total alkaloid, Der Pharmacia Lettre, vol. 3: 226-237.
- Smith, J. and Theresa, J. (2000) Squalene: Potential chemopreventive agent, Expert Opinion on Investigational Drugs, vol. 9: 1841–1848.
- Stein, G. S., Lian, J. B. and Owen, T. A. (1990) Relationship of cell growth to the regulation of tissue-specific gene expression during osteoblast differentiation, FASEB, vol. 4: 3111–3123.

- Stevenson, S. (1985) Bone grafting: Small animal surgery, Edited by: Slatter, D. B, Philadelphia: W. B. Saunders.
- Stevenson, S. (1990) Bone grafting, current techniques in small animal surgery, Edited by Bojrab, J. Philadelphia.
- Stevenson, S., Emery, S. E. and Goldberg, V. M. (1996) Factors affecting bone graft incorporation, Clin Orthop, vol. 66-74.
- Stewart, S. D. A., Shea, C. P., Tarnowski, M. D., Morris, D. and Wang, R. (2002) Trends in early mineralization of murine calvarial osteoblastic cultures: A raman microscopic study, Raman Spectrosc, vol. 33: 536–543.
- Strocchi, R., Orsini, G., Iezzi, A., Scarano, C., Rubini, G., Pecora, A. and Piattelli, J. (2002) Bone regeneration with calcium sulfate: Evidence for increased angiogenesis in rabbits., Oral Implantology, vol. 18: 273.
- Su-Gwam, K., Hak-Kyun, K. and Sung-Chul, L. (2001) Combined implantation of particulate dentine, plaster of paris, and a bone xenograft (bio-oss1) for bone regeneration in rats, CranioMaxillofac Surg Clin North Am, vol. 29: 282–288.
- Tahraoui, A., Hilaly, J., Israili, Z. H. and Lyoussi, B. (2007) Ethnopharmacological survey of plants used in the traditional treatment of hypertension and diabetes in south-eastern morocco (errachidia province), Ethnopharmacology, vol. 110: 105–117.
- Thomas, M. V., Puleo, D. A. and Al-Sabbagh, M. (2005) Calcium sulfate: A review, J Long Term Eff Med Implants, vol. 15: 599-607.
- Thompson, D. D., A. S. , Posner, W. S., Laughlin, a. N. C. and Blumenthal, N. C. (1983) Comparison of bone apatite in osteoporotic and normal eskimos, Tissue Int, vol. 35: 392-393.
- Thomson, R., Yaszemsk, M. J. P., owers, J.M. and Mikos, A.G. (1994) Fabrication of biodegradable polymer scaffolds to engineer trabecular bone, Biomater Sci Polym, vol. 7: 23–38.
- Toriumi, D. M., Kotler, H. S. and Luxenberg, D. P. (1991) Mandibular reconstruction with a recombinant bone-inducing factor. Functional, histologic, and biomechanical evaluation, Arch Otolaryngol Head Neck Surg, vol. 117: 1101-1112.
trabecular bone, Master thesis, University of Lincoln-Nebraska.
- Turner, T. M., Urban, R. M., Gitelis, S., Kuo, K. N. and Andersson, G. B. (2001) Radiographic and histologic assessment of calcium sulfate in experimental animal models and clinical use as a resorbable bone-graft substitute, a bone graft expander, and a method for local antibiotic delivery. One institution's experience, Bone Joint Surg Am., vol. 83: 8-18.
- Turner, T. M., Urban, R. M., Gitelis, S., Sumner, D. R., Haggard, W. O. and Parr, J. E. (1998) Antibiotic delivery from calcium sulfate as a synthetic bone graft, Fourth Combined Meeting of the American and European Musculoskeletal Tumor Societies, vol. 26: 2677–2684.
- Ural, E., Kesenci, K., Fambri, L., Migliaresi, C. and Piskin, E. (2000) Poly(d,lactide/e-caprolactone)/hydroxyapatite composites, Biomaterials, vol. 21: 2147–2154.
- Walters, M. A., Leung, Y. C., Blumenthal, N. C., Legeros, R. Z. and Konsker, K. A. (1990) A raman and infrared spectroscopic investigation of biological hydroxyapatite, Inorg Biochem, vol. 39: 193–200.
- Weiner, S. A. and Wagner, H. D. (1998) The material bone: Structure mechanical function relations, Annu. Rev. Mater. Sci, vol. 28: 271.

- Weiner, S. and Traub, W. (1992) Bone structure: From angstroms to microns, FASEB, vol. 6: 879-885.
- Welch, R. (1996) Effect of rhbmp-2 in tibial fracture models, ACVS Symposium, vol. 13: 136-137.
- Wornom, I. and Buchman, S. (1992) Bone and cartilaginous tissue: Wound healing, Biochemical and Clinical Aspects, vol. 356-383.
- Yamaguchi, M. (1998) Role of zinc in bone formation and bone resorption, Journal of Trace Elements in Experimental Medicine, vol. 11: 119-135.
- Yamaguchi, M. and Yamaguchi, R. (1986) Action of zinc on bone metabolism in rats: Increase in alkaline phosphatase activity and DNA content, Biochem Pharmacol vol. 35: 773-777.
- Yamaguchi, M., Oishi, H. and Suketa, Y. (1987) Stimulatory effect of zinc on bone formation in tissue culture, Biochem Pharmacol, vol. 36: 4007-4012.
- Yamaguchi, M., Oishi, H. and Suketa, Y. (1988) Zinc stimulation of bone protein synthesis in tissue culture, Biochem Pharmacol, vol. 37: 4075-4080.
- Yang, L., Li, Z., Li, X., Wang, Z., Wang, S., Sasaki, Y., Takai, H. and Ogata, Y. (2010) Butyric acid stimulates bone sialoprotein gene transcription, J Oral Sci, vol. 52: 231-237.
- Yasko, A., Lane, J. and Fellingner, E. (1992) The healing of segmental defects, induced by recombinant human bone morphogenetic protein (rhBMP-2) A radiographic, histologic, and biomechanical study in rats, Bone Joint Surg, vol. 74A: 659-670.
- Yasko, A., Lane, J. and Fellingner, E. (1992) The healing of segmental defects, induced by recombinant human bone morphogenetic protein (rhbmp-2) a radiographic, histologic, and biomechanical study in rats, J Bone Joint Surg, vol. 74A: 659-670.
- Yaszemski, M. J., Payne, R. G., Hayes, W. C., Langer, R. and Mikos, A. G. (1996) Evolution of bone transplantation: Molecular, cellular, and tissue strategies to engineer human bone, Biomaterials, vol. 17: 175-185.
- Zioupos, P. (1998) Recent developments in the study of solid biomaterials and bone: "Fracture" And "Pre-fracture" Toughness, Materials Science and Engineering, vol. 6: 33-40.

Appendices

Appendices

Theory of Compression

When a system is squeezed it can be referred to as a compression stressor. Two bones being pressed tighter together is the greatest example of such a case. For example, your femur articulates with the tibia. When a patient stand, the condyles of those bones compressed against each other. This stress is greater when under a load of resistance. If you take a carrot, and bend it, you notice that the highest amount of stress occurs on the outer edges of the root as it is being stretched, and bent (compressed) at the same time. On the inside, the pressures cancel each other out (Marrieb 2001). Therefore when you hear the root snap, it is due to the outside aspects this particular system illustration of why this is the case. It must bare all the weight superior too it, and it is placed at an angel, which allows us to walk fully upright.

Torsion

Supination is the process by which you twist your forearm so as to turn your palm superiorly (facing upwards). Twisting dumbbell curls take advantage of this motion by beginning with the palm turned downwards. As one turns their wrist, the weight is actually resisting in the opposite direction, thus the forearm bones are being twisted in opposite directions at once. This is what is known as torsion. When Marshal Faulk stops and spins the opposite direction, he has stressed his tibia(lower

leg) by exposing it to this force. failing, and not the inner regions (or nowhere near). Weight on your bones, normally occurs off center Meaning, the same effect is occurring. Thus, as wolfs law states, shape is related to function. Therefore you can deduce that the outside of the bone would contain a higher amount of cortical tissue.

Tension

Any type of “ pulling “ force, qualifies as a form of tension. Two main pulling forces act on bones. The first is based on what is called the “ musculotendon unit. This comprises the joining of a tendon into a particular bony region. Thus, the musculotendon unit distally for the biceps brachii is on the lateral bone of the forearm, that is- the radius. As a muscle contracts its force is transferred via this attachment point, and it is during this time where the aspect of insertion is pulled. All forces have four unique characteristics. former refers to the amount of force applied, the latter refers to where the force is applied to. Thus, the level at which the biceps and its line of pull effect the stress level on the bone. Though the insertion point cannot be changed, the line of pull can (Hayes 1986). Keep that in mind, as it has significant application for those attempting to enhance speed and strength. Ligaments also exert a pulling force on the bone. The second main form of tension is opposite that of compression. Simply put, it is the stretching aspect that occurs when you bend a carrot. The femur provides an excellent.

Shearing Stress

When two bones slide past each other, the result, or rather the process is known as shearing. For example, if an athlete bounces on a preacher curl, his forearm will slide downwards while the

humerus remains in place. In football, when an player clips an opponent, their shin bone will slide forward while the thigh remains steady.

Type of bone graft

Autograft

Autogenous cancellous bone graft is the most effective bone graft material possessing all four characteristics. Few mature osteoblasts survive the transplantation but adequate numbers of precursor cells do. It is from these precursor cells that the osteogenic potential is derived. Limitations include the increased operative time, limited availability and significant morbidity related to blood loss, wound complications, local sensory loss and, most importantly, chronic pain donor site pain persisting for more than months has been reported in up to 15% of patients having an iliac graft harvested. The amount of pain seems to be proportional to the extent of dissection required to obtain the graft.

Allograft

Allograft as an alternative offers the same characteristics as autograft with the exclusion of osteogenic cells. It does possess osteoinductive properties but these may not be recognized unless the graft is utilized in either a morsellized or demineralized form. Complications associated with allograft include fracture, non-union and infection.

The advantages of bone allograft recovered from deceased donor sources include its ready availability in various shapes and sizes, avoidance of the need to sacrifice host structures and no

donor-site morbidity. Bone allografts are distributed through regional tissue banks and by most major orthopaedic and spinal companies. Still, the grafts are not without controversy, particularly regarding their association with the transmission of infectious agents, a concern virtually eliminated through tissue-processing and sterilization. However, uncontrolled and unvalidated processing and irradiation protocols may alter graft biomechanical and biochemical properties. It is critical to know your tissue bank provider to ensure their processing and preservation methods do not negatively alter the biomechanical and biochemical properties of the tissues intended for a particular clinical use.

Xenografts

Xenografts are grafts shared between different species. Currently, there are two available sources of xenografts used as bone replacement grafts in periodontics: bovine bone and natural coral. Both sources, through different processing techniques, provide products which are biocompatible and structurally similar to human bone. Recently, porcine bone xenografts have also been described. Xenografts are osteoconductive, readily available and risk free of disease transmission. The latter point has been questioned with the discovery of bovine spongiform encephalopathy, particularly in Great Britain.

Isograft

isograft is material that is taken from one individual and transplanted into another genetically identical individual, such as an identical twin. In this case the donor and the recipient must have the same genotype.

Fourier Transform Infrared (FTIR) Spectroscopy

Fourier transform infrared (FTIR) spectroscopy is an emergent physico-chemical technique, which possesses great sensitivity very small alterations in bond lengths and angles in macromolecules can be detected by this technique and, therefore, it has emerged as a powerful tool to investigate the molecular structural changes in detail.

FTIR spectroscopy allows for the rapid, high-throughput, non-destructive analysis of a wide range of sample types. One advantage of FTIR spectroscopy is that a spectrum from an intact cell can be recorded within a few seconds. Also recent technical advances have made imaging systems available that are able to provide fast FTIR images of tissue sections, requiring only a few minutes to obtain a functional FTIR image of $\sim 1 \text{ mm}^2$ area of tissue. Thus, useful chemical information can be extracted from IR spectra for different pathologies. FTIR has been recognized as a valuable tool for metabolic fingerprinting, owing to its ability to analyse carbohydrates, amino acids, fatty acids, lipids, proteins and polysaccharides simultaneously.

Infrared Spectroscopy

Infrared (IR) spectroscopy is one of the most common spectroscopic techniques used by organic and inorganic chemists. Simply, it is the absorption measurement of different IR frequencies by a sample positioned in the path of a beam.

The main goal of IR spectroscopic analysis is to determine the chemical functional groups in the sample. Different functional groups absorb characteristic frequencies of IR radiation. Using various sampling accessories, IR spectrometers can accept a wide range of sample types such as gases, liquids, and solids. Thus, IR spectroscopy is an important and popular tool for structural elucidation and compound identification.

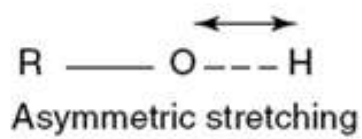
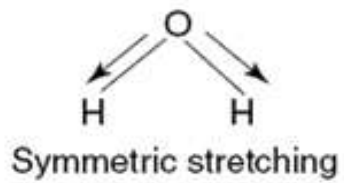
Theory of infrared spectroscopy

The energy of infrared radiation corresponds to energy differences between different vibrational modes in molecules. Infrared spectroscopy is therefore a probe of the vibrational motion of the molecules. It is based on the principle that when a sample is exposed to an infrared (IR) beam, the functional groups within the sample will absorb the infrared radiation and vibrate in one of a number of ways [Figure 1 (a, b)]: either stretching, bending, deformation or combination vibrations. These absorptions/vibrations can then be correlated directly to biochemical species and the resultant infrared absorption spectrum can be described as an infrared ‘fingerprint’ characteristic of any chemical or biochemical substance. Most disease diagnoses researchers have concentrated on the mid-IR part of the spectrum (from 4000–600 cm^{-1}).

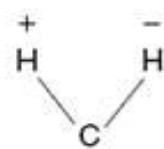
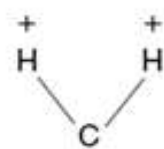
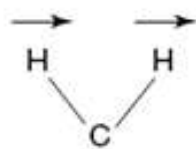
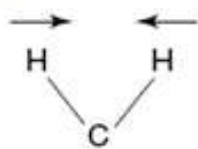
An important issue to be mentioned here is that not all types of vibrations can be detected by IR spectroscopy. Since the technique involves the interaction of electromagnetic radiation with the

molecules only those vibrational transitions which are accompanied by a change in the dipole moment can be detected. This means that diatomic molecules such as O_2 and N_2 do not exhibit infrared absorption and the vibration states of such molecules cannot be examined through IR spectroscopy. This is because the dipole moment is a measure of the separation of the centers of total positive charge and the total negative charge of the molecule. Diatomic molecules can only undergo symmetric vibrations, which will not change the dipole moment. For similar reasons, certain types of vibrations in particular molecules are IR-active, while others are IR-inactive. For example, in carbon dioxide, a symmetric stretching vibration of the oxygen-carbon bonds is IR-inactive, while asymmetric stretching or bending vibrations are IR-active. The principle behind this technique is to irradiate the sample by a sharp pulse of IR radiation that contains all the relevant frequencies and then to detect the response as a function of time. A Fourier transform of this detected signal yields the conventional absorption spectrum.

(a)



(b)



Deformation

Rocking

Wagging

Twisting

Figure 1: (a) Symmetric and asymmetric stretching vibration
(b) Different types of bending vibrations

Raman Spectroscopy

The scattering of light may be thought of as the redirection of light that takes place when an electromagnetic (EM) wave (i.e. an incident light ray) encounters an obstacle or nonhomogeneity, in our case the scattering material (solid, liquid or gas). As the EM wave interacts with the matter, the electron orbits within the constituent molecules are perturbed periodically with the same frequency (ν_0) as the electric field of the incident wave. The oscillation or perturbation of the electron cloud results in a periodic separation of charge within the molecules, this is called an induced dipole moment. The oscillating induced dipole moment is manifest as a source of EM radiation, thereby resulting in scattered light. The majority of light scattered is emitted at the identical frequency (ν_0) of the incident light, a process referred to as elastic scattering. However, as explained below, additional light is scattered at different frequencies, a process referred to as inelastic scattering. Raman scattering is one such example of inelastic scattering. In summary, the above comments describe the process of light scattering as a complex interaction between the incident EM wave and the material's molecular/atomic structure.

Raman Spectroscopy is a non-destructive way of analyzing the molecular structure of the mineral components of bone at the microscopic level. Raman can characterize both the organic and inorganic component of bone by examining the vibrational spectra of the chemical bonds. It provides quantitative information on the changes in the mineral and matrix composition as well as the nature and quantities of mineral constituents. These advantages of Raman Spectroscopy have led to it gaining in importance as a method to study the compositional changes of bone due to aging, diseases and mechanical deformation.

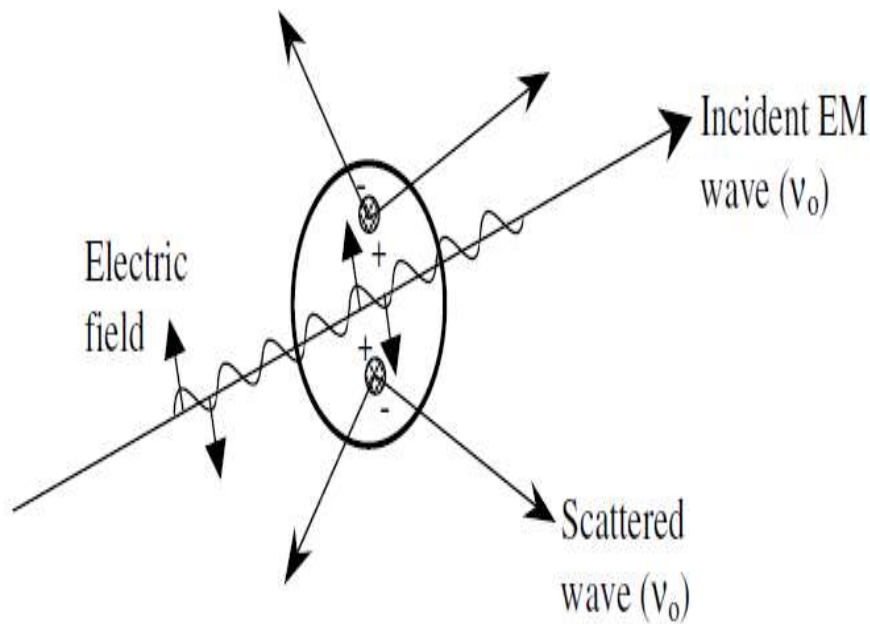
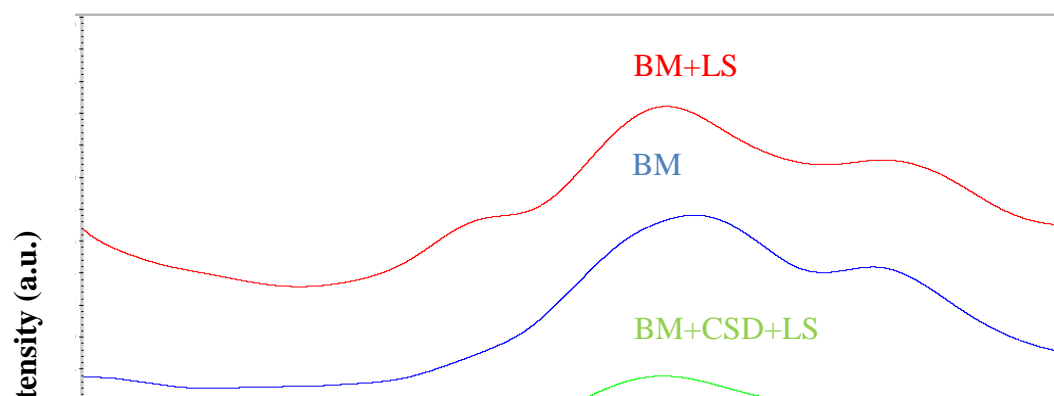
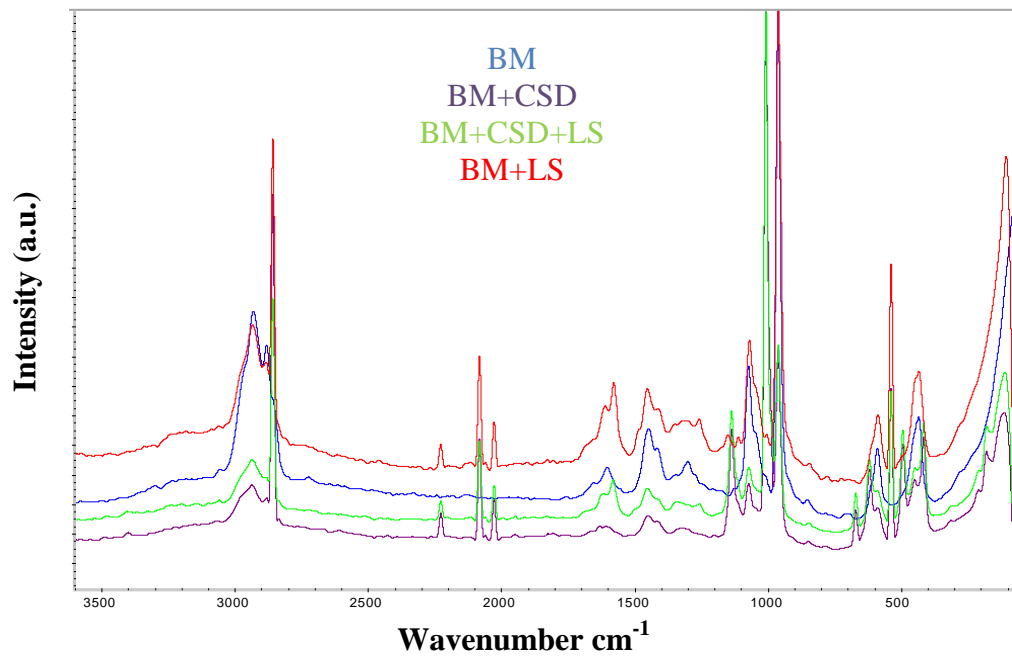


Figure 2: Light scattering by an induced dipole moment due to an

incident EM wave.

By contrast, Raman spectroscopy measures the exchange of energy with electromagnetic radiation of a particular wavelength. This exchange of energy results in a measurable Raman shift in the wavelength of the incident laser light, this shift is complementary to IR absorption and one can also construct a Raman 'fingerprint' of the same sample. Thus Raman and IR spectra provide complementary information. A variety of artificial materials has been used over the centuries to fill bone defects. Synthetic bone grafts at most possess only two of the four characteristics of an ideal bone graft material (osteointegration, osteoconduction). Ideally synthetic bone graft substitutes should be biocompatible, show minimal fibrotic reaction, undergo remodelling and support new bone formation. From a mechanical point of view synthetic bone graft substitutes should have a similar strength to that of the cortical/cancellous bone being replaced. This needs to be matched with a similar modulus of elasticity to that of bone in an attempt to prevent stress shielding as well as maintaining adequate toughness to prevent fatigue fracture under cyclic loading. Synthetic materials that demonstrate some of these properties are composed of either calcium, silicon or aluminum.

Raman Results



BM+CSD

DMA Results

1) BM+CSD

Time min.	Temperatuer C	Strain %	Stress Mpa	Static force N
0.007592	20.15037	-21.5777	6.46E-05	0.001001
0.039167	20.1505	-21.5779	6.47E-05	0.001002
0.0725	20.15029	-21.6457	0.000258	0.004002
0.105833	20.14856	-21.886	0.002055	0.031835
0.139167	20.14712	-21.9212	0.004206	0.065169
0.1725	20.14568	-21.9459	0.006357	0.098502
0.205833	20.14374	-21.9601	0.008508	0.131835
0.239167	20.14346	-21.9694	0.01066	0.165169
0.2725	20.14608	-21.9768	0.012811	0.198502
0.305833	20.14638	-21.9843	0.014962	0.231835
0.339167	20.14666	-21.9907	0.017114	0.265168
0.3725	20.14923	-21.9961	0.019265	0.298502
0.405833	20.15356	-22.001	0.021416	0.331835
0.439167	20.15446	-22.0058	0.023568	0.365168
0.4725	20.15636	-22.0106	0.025719	0.398502
0.505833	20.15756	-22.0155	0.02787	0.431835
0.539167	20.16024	-22.0203	0.030021	0.465168
0.5725	20.16819	-22.0245	0.032173	0.498502
0.605833	20.1714	-22.0283	0.034324	0.531835
0.639167	20.17002	-22.0316	0.036475	0.565168
0.6725	20.16856	-22.0345	0.038627	0.598502
0.705833	20.16614	-22.0371	0.040778	0.631834

0.739167	20.16267	-22.0396	0.042929	0.665168
0.7725	20.15827	-22.0421	0.04508	0.698501
0.805833	20.15396	-22.0446	0.047232	0.731834
0.839167	20.15063	-22.047	0.049383	0.765168
0.8725	20.14864	-22.0492	0.051534	0.798501
0.905992	20.14525	-22.0513	0.053686	0.831834
0.939333	20.14004	-22.0536	0.055837	0.865167
0.972667	20.13852	-22.0558	0.057988	0.898501
1.006	20.13941	-22.058	0.060139	0.931834
Time	Temp	Strain	Stress	Static
0.39333	20.14095	-22.0601	0.062291	0.965168
0.72666	20.14294	-22.062	0.064442	0.998501
0.006	20.14522	-22.064	0.066593	0.001834
1.13933	20.149	-22.066	0.068744	0.065167
1.17266	20.151	-22.068	0.070895	0.098501
0.206	20.153	-22.07	0.073046	0.031834
1.23933	20.156	-22.072	0.075197	0.065167
1.27266	20.159	-22.074	0.077348	0.098501
0.306	20.161	-22.076	0.079499	0.031834
1.33933	20.164	-22.078	0.08165	0.065167
1.37266	20.167	-22.08	0.083801	0.098501
0.406	20.169	-22.082	0.085952	0.031834
0.338	20.64	-32.19076	0.022023	0.226835
0.371	20.65	-32.19547	0.025259	0.260168
0.404	20.66	-32.2013	0.028495	0.293502
0.438	20.69	-32.20776	0.031731	0.326835
0.471	20.72	-32.21497	0.034967	0.360168
0.504	20.74	-32.22246	0.038204	0.393501
0.538	20.76	-32.23045	0.04144	0.426835
0.571	20.77	-32.23824	0.044676	0.460168
0.604	20.77	-32.24588	0.047912	0.493502
0.638	20.77	-32.25368	0.051148	0.526835
0.671	20.78	-32.26134	0.054385	0.560168
0.704	20.77	-32.26906	0.057621	0.593501
0.738	20.77	-32.27641	0.060857	0.626834
0.771	20.79	-32.28322	0.064093	0.660168
0.804	20.8	-32.28896	0.067329	0.693501
0.838	20.8	-32.29432	0.070566	0.726835
0.871	20.8	-32.29968	0.073802	0.760168
0.904	20.8	-32.30462	0.077038	0.793501
0.938	20.81	-32.30926	0.080274	0.826834
0.971	20.82	-32.3138	0.08351	0.860168
1.004	20.82	-32.31882	0.086747	0.893501
1.038	20.83	-32.32383	0.089983	0.926834
1.071	20.84	-32.32887	0.093219	0.960168
1.105	20.84	-32.3338	0.096455	0.993501
1.138	20.84	-32.33815	0.099691	1.026834
1.171	20.83	-32.34221	0.102928	1.060168
1.205	20.82	-32.34607	0.106164	1.093501

2) B

M+CSD+

LS

1.238	20.82	-32.34952	0.1094	1.126834
1.271	20.81	-32.3531	0.112636	1.160168
1.305	20.8	-32.35681	0.115872	1.193501
1.338	20.79	-32.36021	0.119109	1.226834
1.371	20.79	-32.36336	0.122345	1.260167
1.405	20.78	-32.36643	0.125581	1.293501
Time 1.438	Temperature 20.77	Strain 32.36963	Stress 128817	Static 1.26834
min. 1.471	C 20.75	% 32.3727	Mpa 32053	N 360167
0.001125	19.74298	-5E-05	6.76E-05	0.001003
0.025333	19.74344	-6.5E-05	6.76E-05	0.001003
0.058667	19.7418	-0.00011	6.76E-05	0.001003
0.092	19.73861	-3.3E-05	6.76E-05	0.001003
0.125333	19.7346	6.02E-05	6.76E-05	0.001003
0.158667	19.72938	2.55E-05	6.76E-05	0.001003
0.192	19.72538	1.59E-05	6.76E-05	0.001003
0.225333	19.72316	4.25E-05	6.76E-05	0.001003
0.258667	19.72009	-1.4E-05	6.76E-05	0.001003
0.292	19.71708	8.46E-06	6.76E-05	0.001003
0.325333	19.71464	2.25E-05	6.76E-05	0.001003
0.358667	19.7133	-2.7E-05	6.76E-05	0.001003
0.392	19.71048	-0.00011	6.76E-05	0.001003
0.425333	19.70852	2.18E-05	6.76E-05	0.001003
0.458667	19.70856	-4.8E-06	6.76E-05	0.001003
0.492	19.70866	-4.2E-05	6.76E-05	0.001003
0.525408	19.7059	9.2E-06	6.76E-05	0.001003
0.558833	19.70055	-9.3E-06	6.76E-05	0.001003
0.592167	19.69716	2.47E-05	6.76E-05	0.001003
0.6255	19.69353	-8.5E-06	6.76E-05	0.001003
0.658833	19.69031	-1.9E-06	6.76E-05	0.001003
0.692167	19.68753	-1.7E-05	6.76E-05	0.001003
0.7255	19.68548	-6.6E-05	6.76E-05	0.001003
0.758833	19.68459	-4.3E-05	6.76E-05	0.001003
0.792167	19.68182	-8.5E-06	6.76E-05	0.001003
0.8255	19.68081	-4.4E-05	6.76E-05	0.001003
0.858833	19.67899	-4.6E-05	6.76E-05	0.001003
0.892167	19.67702	-2E-05	6.76E-05	0.001003
0.9255	19.67353	1.59E-05	6.76E-05	0.001003
0.958833	19.67074	7.5E-05	6.76E-05	0.001003
0.992167	19.66924	3.21E-05	6.76E-05	0.001003
1.0255	19.67063	-2.7E-05	6.76E-05	0.001003
1.058833	19.67319	-0.00011	6.76E-05	0.001003
1.092167	19.67598	-0.0002	6.76E-05	0.001003
1.1255	19.6772	-0.00014	6.76E-05	0.001003
1.158834	19.67749	-0.00013	6.76E-05	0.001003
1.192167	19.67996	-0.00017	6.76E-05	0.001003
1.2255	19.68227	-0.00017	6.76E-05	0.001003
1.258833	19.68246	-0.00014	6.76E-05	0.001003
1.292167	19.68247	-0.00021	6.76E-05	0.001003

3) B

M+ LS

1.3255	19.6801	-0.00019	6.76E-05	0.001003
1.358833	19.67903	-0.00013	6.76E-05	0.001003
1.392166	19.67908	-0.00016	6.76E-05	0.001003
1.4255	19.67612	-0.00018	6.76E-05	0.001003
1.458833	19.67572	-9.7E-05	6.76E-05	0.001003

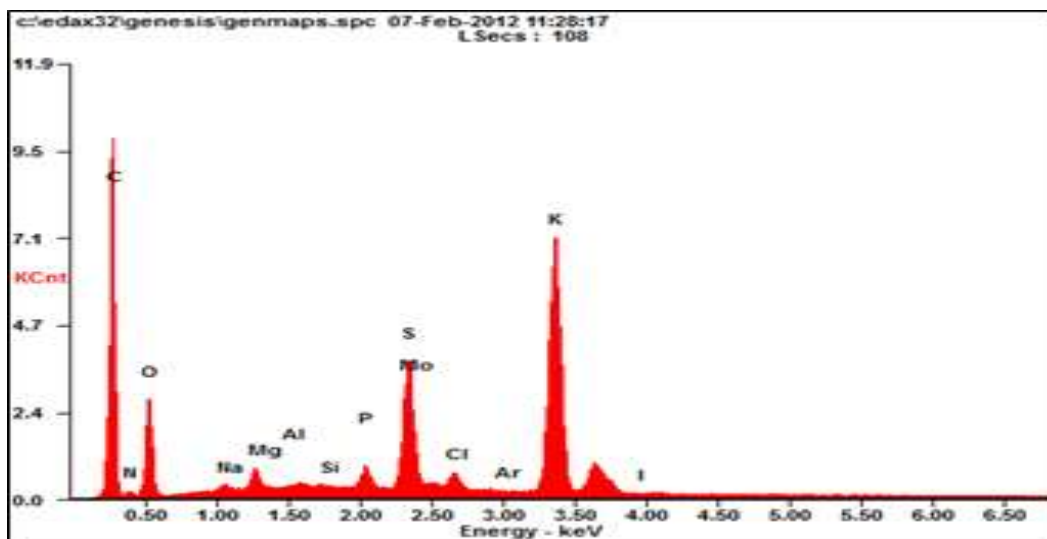
GC/MS

Results

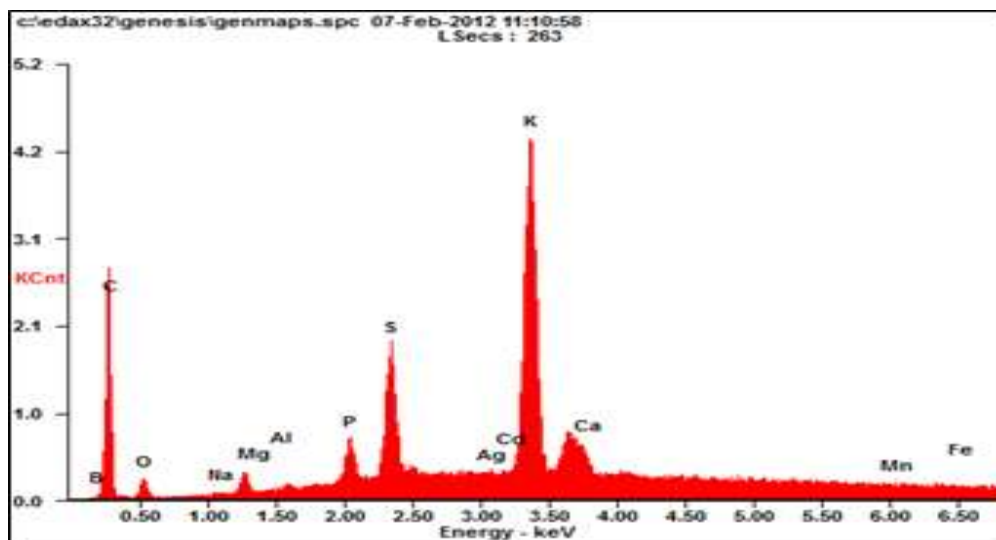
MIN	AREA
5.221	240195.1
5.241	54254.8
5.411	634461.4
5.571	6498138
5.737	240525.3
5.802	2820786
6.067	24835.83
6.183	10421051
6.258	80510.48
6.278	210809.5
6.353	3749667
6.563	1.39E+08
6.809	2886337
6.989	508017.7
7.23	986960.4
8.507	818046.7
8.602	745642.6
8.803	466815.8
8.838	17056.61
8.908	512753.6
9.168	12608.95
9.223	16497926
9.399	350810.8
9.895	277775.5
10.391	3884.693
10.496	359882.6
10.771	276078.3
11.257	740020.4
11.648	275712.7
12.024	245724.2
12.059	7872.208
12.184	207235.9
12.214	27559.62
12.264	193044.2
12.87	799490.4
13.011	664294

13.111	634063.9
13.211	294526.2
13.331	1830.264
13.391	271913.5
13.482	1598024
13.832	1115402
14.068	586121.6
14.188	823371.6
14.238	174859.4
14.283	173265.1
14.548	838528.1
14.679	2334.263
14.804	371555.1
15.059	3569.079
15.12	477195
16.733	14858676
16.783	72989.31
16.878	816448.5
16.958	3999.226
17.023	1506196
17.113	350881.2
17.444	405572.1
17.589	192709.2
17.815	1203115
18.015	162798.6
18.246	423090.4
18.426	290642
18.596	68633272
18.696	610811.2
19.152	781678.3
19.393	2419135
19.428	1389997
19.548	3952960
19.573	1358998
19.703	2553963
19.758	7846413
19.784	1322700
19.839	2450180
19.949	1696298
20.044	6399948
20.114	229395
20.189	4142143
20.375	1229518
20.42	170566.9
20.48	277052.8
20.5	1389957
20.62	3915173
20.68	356996.5
20.765	4448912

20.861	244416.8
20.901	1021595
21.011	1062148
21.432	176539.9
21.467	203278.9
21.542	493490.8
21.582	16734.71
22.454	1246245



<i>Element</i>	<i>Wt%</i>	<i>At%</i>
<i>CK</i>	45.87	61.81
<i>NK</i>	02.75	03.18
<i>OK</i>	21.59	21.84
<i>NaK</i>	00.44	00.31
<i>MgK</i>	01.03	00.68
<i>AlK</i>	00.09	00.05
<i>SiK</i>	00.09	00.05
<i>PK</i>	00.94	00.49
<i>MoL</i>	00.00	00.00
<i>SK</i>	06.60	03.33
<i>ClK</i>	00.99	00.45
<i>ArK</i>	00.00	00.00
<i>KK</i>	18.49	07.65
<i>IL</i>	00.15	00.02
<i>CoK</i>	00.06	00.02
<i>CuK</i>	00.21	00.05
<i>ZnK</i>	00.00	00.00
<i>AuL</i>	00.30	00.02
<i>HgL</i>	00.42	00.03
<i>Matrix</i>	Correction	ZAF



<i>Element</i>	<i>Wt%</i>	<i>At%</i>
<i>BK</i>	01.82	03.22
<i>CK</i>	40.94	65.01
<i>OK</i>	05.83	06.96
<i>NaK</i>	00.15	00.12
<i>MgK</i>	01.32	01.04
<i>AlK</i>	00.10	00.07
<i>PK</i>	02.38	01.47
<i>SK</i>	08.08	04.81
<i>AgL</i>	01.67	00.29
<i>CdL</i>	01.31	00.22
<i>KK</i>	28.59	13.95
<i>CaK</i>	03.72	01.77
<i>MnK</i>	00.35	00.12
<i>FeK</i>	00.40	00.14
<i>CuK</i>	02.03	00.61
<i>ZnK</i>	00.39	00.11
<i>AuL</i>	00.91	00.09
<i>Matrix</i>	Correction	ZAF

