Indonesian Journal of
Tropical and Infectious Disease
Vol. 5. No. 2 May–August 2014

Research Report

COLOSTRUM-COLLAGEN-HYDROXYAPATITE COMPOSITE, AN EXCELLENT CANDIDATE BIOMATERIAL FOR BONE REPAIR AND BONE INFECTION MANAGEMENT

Dio Nurdin Setiawan[1], Mirzaq Hussein Anwar[1], Kholifatul Wanda Putri[2], Nilna Faizah Fiddarain[3], Prihartini Widiyanti[4,5], Heri Purnobasuki[6]

[1] Bachelor of Biomedical Engineering Study Program, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia
[2] Bachelor of Information System Study Program, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia
[3] Bachelor of Pharmacy Study Program, Faculty of Pharmasy, Universitas Airlangga, Surabaya, Indonesia
[4] Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia
[5] Biomedical Engineering Study Program, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia
[6] Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia

ABSTRACT

In the case of bone fracture or defect after surgery, which is common in patients with bone cancer (osteosarcoma), it takes a long time for closure and it may cause an infection problem. The use of collagen-hydroxyapatite composite with a blend of colostrum as a scaffold is aimed to accelerate the process of osteoblast growth, inhibite the emergence of infections, and act as bone tissue repair material. The method used was the hydrogel formation process and freeze dry process to remove the solvent and to form pores. The composition of scaffold composite manufactured was 15% collagen, 75% hydroxyapatite and 10% colostrum. Combination of scaffold collagen-hydroxyapatite-colostrum has quite reliable properties because SEM test showed that scaffold could bind to both and could bind to both and could form sufficient pores to provide enough place for bone cells (osteoblats) to grow. The results of MTT assay revealed percentage of above 60%, which indicates that the material is not toxic. In conclusion, collagen-hydroxyapatite-colostrum combination is an excellent biomaterial candidate for bone repair and bone infection management.

Key words: *collagen, hydroxyapatite, colostrums, osteoblasts, bone repair*

ABSTRAK

Pada kasus fraktur atau defek tulang setelah operasi yang biasa terjadi pada penderita osteosarkoma (kanker tulang), membutuhkan waktu yang lama dan bisa menimbulkan problem infeksi. Penggunaan komposit kolagen-hidroksiapatit dengan paduan colostrums sebagai scaffold diharapkan dapat mempercepat proses pertumbuhan sel osteoblast. Metode yang digunakan yaitu dengan proses freeze dry untuk menghilangkan pelarut dan membentuk pori. Perbandingan pembuatan komposit scaffold ini 15% kolagen, 75% hidroksiapatit, dan 10% colostrums. Paduan colostrums scaffold Kolagen-Hidroksiapatit memiliki sifat cukup baik karena pada hasil uji SEM scaffold dapat berikatan dengan baik dan dapat terbentuk pori yang cukup untuk tumbuhnya sel tulang (osteoblast). Hasil MTT Assay menunjukkan jumlah sel hidup diatas 60% yang berarti bahwa material tidak bersifat toksik.

Kata kunci: *kolagen, hidroksiapatit, kolostrum, osteoblas, perbaikan tulang*

INTRODUCTION

According to World Health Organization (WHO), traffic accidents cause about 1.2 million deaths each year. Losses due to traffic accidents, in addition to death, are physical damage as well. Physical damage most often occours in an accident is fracture (broken bone). High accident rate results in high fracture incidence. Fracture is a situation where bone disintegrating. The most common cause is accidents, but other factors, such as degenerative processes, can also affect the incidence of fracture.

Scaffold is one component of tissue engineering applications that can be used as application in bone tissue repair.[1] In producing scaffold, we require hydroxyapatite (HA). Hydroxyapatite it self has osteoconductive and biocompatibility properties.[2] However, HA also has characteristics of brittle and fatigue failure, so that in health applications HA is only used for unloading bearing repair and as a substitute.[3] In scaffold formation, we need mixed materials for quality enchancement, and the collagen. Approximately 25–35% of body proteins are composed by collagen.[3–6,]

In the case of fracture or bone defect after surgery, which is common in patients with bone cancer (osteosarcoma), it takes a long time for closure. To overcome this problem, additional material other than HA and collagen is required to accelerate the regeneration of bone cells. Regeneration of bone cells is also affected by immune quality of the human body. Self-immunity is provide by many living things, including mammals. There is a fact in the society that drinks containing colostrums can accelerate healing, especially in adult to elderly whose healing process requires longer time.

In this study, we added bovine colostrums to collagen-HA scaffold. Bovine colostrums has content which is almost similar to that of human colostrums. Colostrums itself has properties to stimulate body cells regeneration, peptide imunotheraphy, help fighting viruses, and so on.[7] We expect that a combination of blend collagen-HA scaffolds and colostrums may accelerate cell regeneration, so that it may implicate the acceleration of bone grafting.[2,4]

MATERIALS AND METHOD

Materials that used in this research were hydroxyapatite of bovine obtained from Tissue Bank and Biomaterial Center Dr. Soetomo General Hospital Surabaya, East Java, Indonesia. Collagen powder is derived from the skin of bovine, and colostrums powder is derived from dairy cattle.

Preparation of Collagen-Hidroxyapatite Addition Colostrums. 15% collagen dissolved in 0.5 mol/L cold acetic acid, then added with Na_2HPO_4 0.02 mol/L and controlling pH up to 7.2 with aqueous NaOH solution at temperature below 10°C. Then collagen solution was added 75% hydroxyapatite are stirred in NH_4OH for 2 hours, at pH 7. After dissolved, add 10% colostrums and they were stirred for 4 hours, and then incubated at a temperature of 35°C for 20 hours. The results scaffold obtained, then washed with aquadest, and then centrifuged, thus a mixture is obtained in the form of hydrogel and done printing. Solid phase separation technique and liquid is done with composite cooling up to –20°C for 24 hours, while the solvent removed by freeze-drying. Characterizations were carried out with Scanning Electron Microscope (SEM). Biocompatibility was tested by MTT Assay.

RESULTS AND DISCUSSION

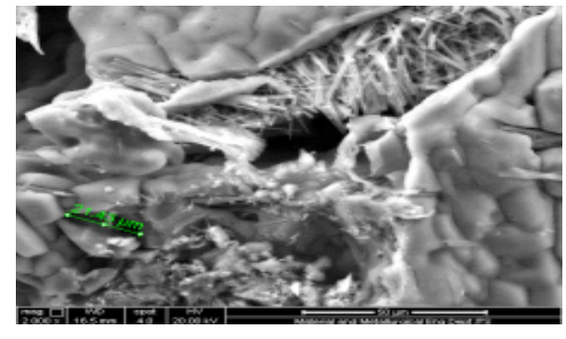
In collagen - HA - colostrums composite facilitate interaction between cells and implants material. It will cause the occurrence of osteogenic cells, adhesion, attachment and spreading phase which triggered the proliferation and differentiation of cells. Attachment phase and physicochemical linkage formed appear at the same time with biomaterial implantation to bone cells.[8] Adhesion and spreading phase will occur when focal contacts and adhesion plaque between the surface of the implant material and cell membrane is formed. Then actin filament reorganized cause adhesion process causes change cell and transmit signal transduction through proteins of the cytoskeleton to become nuclear matrix, changing the arrangement of the genes, and determine the number of cells for proliferation and differentiation.[9]

Composite of collagen-HA as bone tissue engineering should have physichocemical binding and crystal structure to be recognized as good material. These will affect the characteristic of the osteogenic cells, determine the quality of them and success to perform new bone tissue. Calcium phosphate powders is required on the average diameter 200–500 m, and if the size of particles less than 50 m then it will cause cytotoxicity.[10] The pore size which are ideal for calcium phosphate is around 200-400 m. It provide the space for blood vessels and trigger migration, adhesion, proliferation, and differentiation of osteoblast in pores.

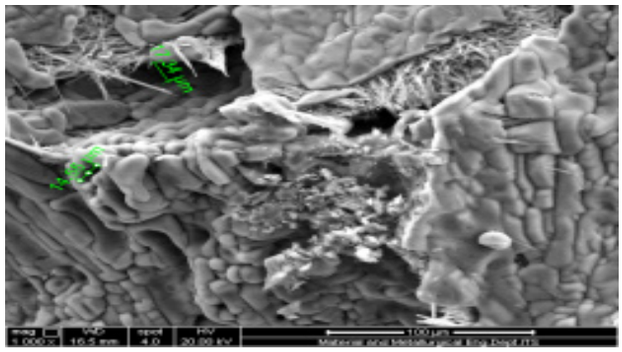
The results of this research material products could be seen in Figure 1.

Figure 1. Scaffold Collagen – HA – colostrums

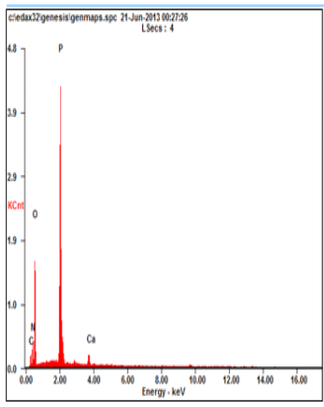
SEM profile with EDAX in Figure 2 below shows that the third main material in this research have been well-mixed.



(a)



(b)



Element	Wt%	At%
CK	09.10	13.12
NK	17.08	21.13
OK	47.09	50.99
PK	25.32	14.16
CaK	01.40	00.61
Matrix	Correction	ZAF

(c)

Figure 2. Result (a) SEM with magnification 2000×, (b) SEM with magnification 1000×, (c) Energy X-Ray Dispersion.

SEM results are showed shape of HA grain and collagen fibers. While the fiber shape of colostrum is already bind to HA. MTT assay is showed that the material biocompatibility exceeds 50% by the procentage of living cell.[11,12]

Combination of colostrum on scaffold collagen - hydroxyapatite is showed good physical properties. this can be evidenced from the test results the main material of the SEM, result can bind to either and form a good pore as a condition the growth of osteoblast cells. Interpretation of MTT assay result which exceed 60% could be considered that the material is not toxic. Colostrum can actively support the body immunity, antibodies, and other protective proteins. Colostrum provides 'first immunity' and the mechanism is to protect the body against many infections. The main immunity substances are immunoglobulin that can prevent and fight bacteria, viruses, fungi and toxins. Immunoglobulin (IgA) was act as the protectors in the area susceptible to bacteria commonly in the membranes of lung, colon and throat. Colostrum contains white blood cells (leukocytes) which its function could fight microorganism. Colostrum contains growth factors which could accelerate wound healing. Colostrum which is rich in vitamins A and E will support to reduce infection. In addition, Colostrum also contains vitamin B6, B12, C, D, K and minerals, especially iron and calcium. Colostrum also contains several substances in such high quantities of sodium, potassium and cholesterol.[13] Based on the phenomena above, a blend of collagen-hydroxyapatite-colostrum is a candidate for an promising biomaterial for bone repair and bone infections treatment.

CONCLUSION

The composition of scaffold composite manufactured was 15% collagen, 75% hydroxyapatite and 10% colostrums has quite reliable properties because SEM test showed that scaffold could bind to both and could bind to both and could form sufficient pores to provide enough place for bone cells (osteoblats) to grow. The results of MTT assay revealed percentage of above 60%, which indicates that the material is not toxic. So that collagen-hydroxyapatite-colostrum combination is an excellent biomaterial candidate for bone repair and bone infection management.

REFERENCES

1. Cahyanto A. 2009. Biomaterial. Departemen Ilmu dan Teknologi Material Kedokteran Gigi. Universitas Padjadjaran. Bandung.
2. Feng, W. Tang, K. Zheng, X. Yuanming. Liu, J. 2009. Preparation and Characterization of Porous Collagen/Hydroxyapatite/Gum Arabic Composit. Zhengzou University: Cina.
3. Rodrigues CVM. 2003. Characterization of Bovine Collagen-Hydroxyapatite Composite Scaffold for Bone Tissue Engineering. Biomaterials, 2003; 24: 4987–4997.
4. Gelse, KE. Poschl, T. Aigner. 2003. Collagens-Structure, Function, and Synthesis. Advanced Drug Delivery, 2003; 55: 1531–1546.
5. Lawson AC, Czernuszka JT, 1998. Collagen–calcium phosphate composites. Proc Instr Mech Eng, 1998; 212 (11): 413–438.
6. Song, Eun, So Yeon Kimb, Taehoon Chunc, Hyun-Jung Byunc, Young Moo Lee. 2006. Collagen scaffolds derived from a marine source and their biocompatibility. Biomaterials, 2006;27: 2951–2961.
7. Keech AM. 2009. Peptide Imunotherapy Colostrums. AKS Publishing; ISBN 978-0-692-00242-1.
8. Jie, Wei, Li Yubao. 2004. Tissue engineering scaffold material of nano-apatite crystals and polyamide composite. European Polymer Journal 2004; 40: 509–515.
9. Park JB, Bronzino JD, 2003. Biomaterials Principles and Applications. CRC Press: Boca Raton.
10. Kutz, Myer. 2003. Standard Handbook of Biomedical Engineering and Design. McGraw-Hill: New York.
11 Tierney CM, Haugh MG, Liedl J, Mulcahy F, Hayes B, O'brien FJ, 2009. The effect of Collagen Concentration and Crosslink Density on Biological, Structural and Mechanical Properties of Collagen-GAG Scaffolds for Bone Tissue Engineering. Journal of the Mechanical Behaviour of Biomedica Materials, 2009; 2 (2): 202–9.
12. Wahl DA, Czernuska JT. 2006. Collagen-Hydroxyapatite Composites for Hard Tissue Repair. European Cells nd Materials, 2006; 11: 43–56.
13. Hurley WL. Theil PK. 2011. Perspectives on Immunoglobins in Colostrum and Milk. Nutrients, 2011; 3: 442–474.