Development of a Multifunctional Biomaterial: Bone Graft and Drug Delivery Matrix

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Introduction.

Collagen represents the most abundant animal protein. It has three important characteristics as a biomaterial: low levels of allergenicity; the capability of reorganization in the original structure, as in the native tissue with similar properties, and work as a native protein drug delivery matrix. The mineral tissue of bovine bone (natural hydroxyapatite) plays an important involvement in regenerative tissue process as a required graft material to restore its original structure and functions where bone defects cannot be self-repaired. The purpose of this work is present a possible processing route to obtain a bone graft biomaterial composed by natural hydroxyapatite (NHA) particles coated by a collagen layer that allows to be used as well as drug delivery matrix or a marking to sinovectomy or hepatoma therapy in nuclear medicine.

Materials and Methods.

The developed collagen derived from bovine bone is obtained by chemical procedures in three fundamental steps: (a) demineralization of bovine cortical bone in chloridric acid solution; (b) dissolution of the cortical bone collagen in acetic acid solution and (c) correction of pH with ammonium hydroxide solution.

The NHA particles are obtained from bovine cortical bone structure in the following sequence: (a) cleaning in oxygen peroxide 130 volume; (b) dissolution of contained collagen in acetic acid solution; (c) neutralization of pH in calcium carbonate solution; (d) drying at 80 ^{o}C; (e) grinding in wiley type mill and (f) particle size range classification through a sieve set.

Coating Assay: The coating process of the NHA particles utilized a fluidized bed reactor (FBR) with a collagen solution aerosol spray wetting the mineral particles followed by a hot air flow to dry this blend. A scheme of this procedure is presented in Figure 1.

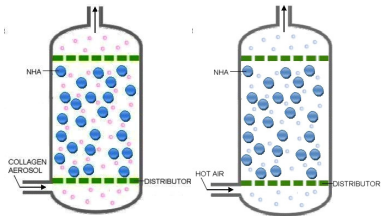

Fig. 1. FBR: (a) coating of hydroxyapatite particles with collagen; (b) drying the coated particles.

Hygroscopicity Assay: The amount of liquid drug vehicle (LDV) absorbed by the collagen layer of the coated NHA particles was measured by the following procedure: (a) water simulating LDC; (b) all samples were dried and weight; (c) the samples were immersed in LDV during 30 minutes, (d) the excess of LDV was removed from the samples by a filter paper and (e) were obtained their wet weights. The absorbed LDV was calculated by the equation 1:

$$\%\,\mathrm{LDV} = \frac{(m_w - m_d)}{m_w} \times 100 \qquad (1)$$

m_w and m_d are the wet and dried weights, respectively.

Cytotoxicity Assay: Carried out by neutral red uptake methodology, utilizing samples extracts dilutions in contact with NCTC L929 cell culture in 96 wells microplates. The medium culture was Minimum Essential Medium (MEM) supplemented with 5% Calf bovine serum. Extract: immersion of samples in MEM for 24h at 37°C. *Controls:* positive (0,02% phenol solution) and negative (no toxic PVC pellets). The cell viability was measured incorporating neutral red by viable cells and in the final procedure cell rupture and optical definition measurements on 540nm filter of spectrophotometer Sunrise – Tecan ELISA reader.

Results and Discussion.

The obtained results of absorbed LDV by the coated NHA particles are representative of five independent measurements as shown in Table I.

Table I. Absorbed LDV by coated NHA particles.

Sample	1	2	3	4	5	%LDV Mean value
m_w (g)	1.70276	1.85756	1.92194	1.93710	1.85430	
m_d (g)	0.48588	0.49909	0.50863	0.48504	0.50736	
% LDV	71.465	73.132	73.535	74.960	72.639	73.15±1.28

The LDV absorption capacity of the NHA particles can be seen in the Figure 2 where it is observed the thickness of collagen layer increases up to at least 2 times its original dried dimension.

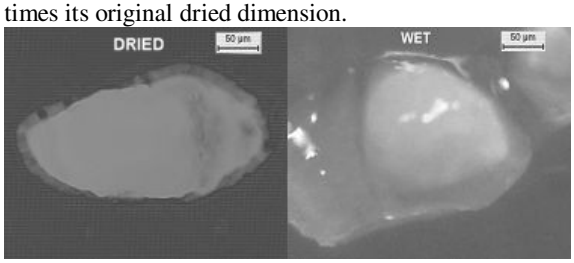

Fig. 2 - LDV absorption of the collagen layer.

The coated NHA particles show viability curve above IC50% line and are considered non-cytotoxic (Figure 3).

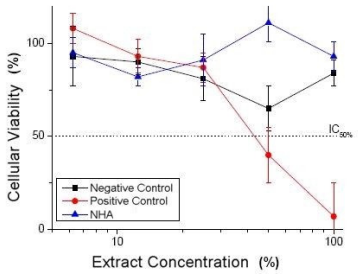

Fig. 3 - Viability curve of coated NHA particles in the in vitro cytotoxicity assay.

Conclusions:

(a) The coating process using a fluidized bed reactor to create a collagen layer over natural hydroxyapatite suggest be appropriated;

(b) The coated natural hydroxyapatite particle has demonstrated be capable to absorb liquid drug vehicle more than 70% of its weight.