Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl20

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Available online: 14 Feb 2012

To cite this article: T. A. du Plessis, G. O. Phillips & S. Al-Assaf (2012): Radiation Modification of Natural Polymers to Enhance Structure and Bioactivity, Molecular Crystals and Liquid Crystals, 555:1, 225-231

To link to this article: http://dx.doi.org/10.1080/15421406.2012.635546

Mol. Cryst. Liq. Cryst., Vol. 555: pp. 225–231, 2012

ISSN: 1542-1406 print/1563-5287 online
DOI: 10.1080/15421406.2012.635546

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Radiation Modification of Natural Polymers to Enhance Structure and Bioactivity

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A technique was developed for the radiation crosslinking of lyophilised demineralised bone matrix (DBM). The crosslinked demineralised bone matrix (DBM) shows a marked enhancement in osteogenic potential in both the rate of new bone formation and the strength of the ensuing new bone. A further advantage of the crosslinking of the DBM* substrate in crosslinked collagen, is its increased mechanical strength and relative insolubility, ensuring it does not wash away upon implantation. The dry crosslinked DBM* and collagen mixture is stored under inert nitrogen gas, eliminating oxidative degradation and obviating the need to transport and store the product under refrigerated conditions, resulting in a shelf-life of at least five years.*

Keywords Bone growth; natural polymers; osteoinductivity; radiation

Introduction

The radiation modification of end-products made from synthetic polymers has been in use very successfully in industry for the past five decades. Similarly, the radiation crosslinking of orthopaedic prostheses made from ultra-high molecular weight polyethylene (UHMWPE) has been in use in South Africa since 1975 [1–4] and since 1998 on a more general scale worldwide [5]. It is estimated that most of the utilised bearing surfaces of UHMWPE acetabular cups today are radiation crosslinked [6]. Clinical case studies in South Africa demonstrated that such UHMWPE acetabular cups radiation crosslinked in the presence of an alkyne mediating gas, could last at least 34 years [7]. More recently, a process for the gamma-radiation modification of feedstock polyethylene was developed in which the polymer is radiation-modified *prior* to the conversion thereof into the end product. In the particular case of polyethylene it was found that with a decrease in the melt flow of the polymer with an increase in the radiation dose, the spiral flow of the polymer remains virtually constant. The radiation-induced oxidation of the polymer, furthermore, renders very good adhesive properties to the modified polymer [8].

Emanating from the particular success achieved over more than three decades with the radiation crosslinking of polyethylene acetabular cups in the presence of a mediating gas, the

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specific technique was applied to a wide range of dry polysaccharides and proteins, as well as some interactive blends, in order to modify their functionalities [9,10]. Demineralised bone matrix (DBM) protein was investigated because of its known capacity to actively induce new bone growth in oral and maxillofacial surgery [11]. The bone biology and the role of DBM in new bone formation were discussed in detail earlier [10].

The radiation modification of the DMB and its potential to enhance the osteoinductive capacity (rate of bone formation) of the protein, as well as the strength of the newly formed bone, were the main aims with this investigation. Similarly, the radiation crosslinking of dry collagen as a carrier for the DBM was investigated in order to present a novel system for bone growth stimulation in surgery.

Experimental

Experimental Developments and Radiation Procedures

The experimental procedures for the preparation and gamma radiation modification of the two proteins were discussed in detail in the associated patent [12]. Hypodermic syringes made from a specially developed radiation-resistant polypropylene were filled with the lyophilised DBM and collagen after replacing the air with acetylene gas as the mediating gas. The proteins were subsequently exposed to gamma irradiation at the Isotron SA industrial irradiator in South Africa, covering the dose range of 4 kGy to 40 kGy. In the case of both the DBM and the collagen, experimental analyses indicated that the two proteins undergo an optimum radiation modification at the same minimum absorbed dose of 16 kGy. This finding has the important implication that the two lyophilised proteins could be mixed in the appropriate compositions in syringes *prior* to the radiation modification and sterilisation thereof - greatly enhancing the simplicity of the preparation procedures and the associated process and sterility assurances of the ensuing bone growth system, and the dispensing thereof in theatre.

Samples of the lyophilised DBM and collagen in hypodermic syringes were radiation modified in the presence of the mediating gas and subsequently packaged under analytical nitrogen gas and hermetically sealed—thus removing any residual acetylene gas. The primary packaging was subsequently hermetically sealed under air and gamma sterilised at a minimum absorbed dose of 25 kGy. Following the filling of the syringes with the lyophilised DBM, the demineralised bone was thus not again exposed to the environment with the associated sterility assurance. It thus obviates the need for the aseptic preparation and handling of the DBM and collagen. Similarly, separate syringes were filled with the appropriate volumes of pyrogen-free sterile water for the reconstitution of the crosslinked demineralised bone matrix (DBM*) prior to the dispensing thereof.

Laboratory Animal Assays on Bone Strength

The radiation modified and sterilised demineralised bone (DBM*) in the initial syringes were subsequently subjected to in vivo rat studies at the tissue bank of Korea. Identical samples that were not exposed to the mediating gas were used as control samples. Similarly, syringes filled with pyrogen-free sterile water and subsequently exposed to the gamma-radiation sterilisation process, were prepared for the reconstitution of the lyophilised and radiation modified mixtures of the two proteins. The details of the rat studies were described earlier [10].

Alkaline Phosphatase Assays as Indicator of Osteoinductivity

The osteoinductivity (rate of bone formation) of the crosslinked (DBM*) versus non-crosslinked (DBM) demineralised bone, alkaline phosphatise assays (ALP) were performed in using the in vivo rat implantation model. These studies were undertaken at the University of Pretoria, the Witwatersrand University and the Tshwane University of Technology [13], whereas assays of cell growth in vitro were undertaken at the University of California, Berkeley [14].

Development of an Administration Set

Many of the existing bone growth stimulants have to be prepared in theatre under aseptic conditions before administration to the operation site. Considering the unique features that the current system offers, a customised administration set was developed for the current bone growth system as presented in Fig. 1. [15].

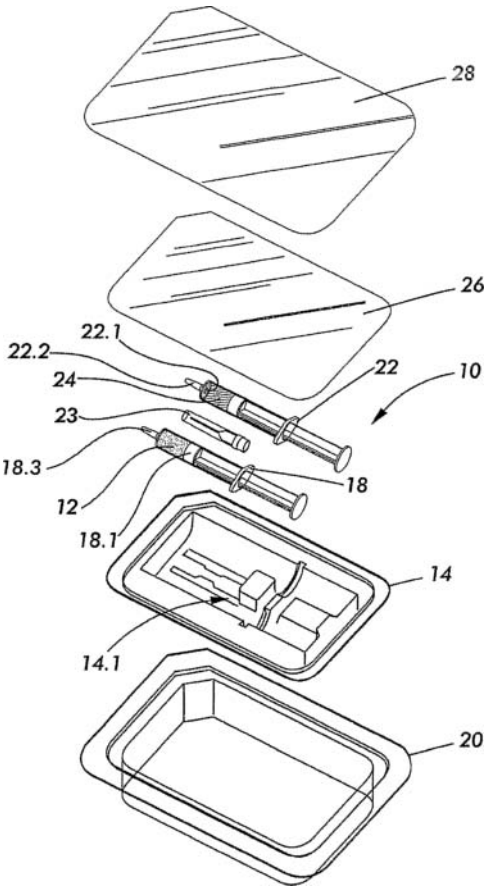

Figure 1. Delivery system for the crosslinked demineralised bone/collagen mixture to operation site. 12 - crosslinked osteoinductive agent, 14 - primary blister, 20 - secondary blister, 18 - syringe for osteoinductive agent, 22 - syringe for sterile water, 23 - cannula, 28 and 26 - peel able lidding for primary and secondary blisters [15].

The lyophilised DBM and collagen mixture is added in the appropriate volumes to the hypodermic syringes—the latter fitted with hubs with a hole where the needle is generally fitted in. The syringes filled with the DBM are subsequently packaged in customised trays, evacuated and hermetically sealed in pouches under die mediating gas. The trays filled with the syringes are subsequently irradiated to a minimum absorbed gamma irradiation dose of 16 kGy.

Upon the completion of the radiation modifying process, the syringes with the DBM* are fitted into a customised primary blister, together with a second syringe containing the appropriated volume of pyrogen-free sterile water for the hydration of the osteoinductive system, and closed with a hub. In addition a cannula, to transfer the water from the one syringe to that containing the osteoinductive mixture, is fitted into the primary blister. The primary blister is then evacuated to remove all air and any residual mediating gas, filled with high-purity nitrogen, hermetically sealed and uniquely labelled. The filled primary blister is subsequently hermetically sealed under air in a secondary blister and packaged in a cardboard shelf container with the necessary unique labelling. This doubly hermetically sealed system is then exposed to a minimum absorbed gamma irradiation dose of 25 kGy as a terminal sterilisation procedure.

To use the set, the secondary and primary blister seals are peeled off in theatre, the cannula fitted to the syringe containing the water and the water injected into the syringe with the osteoinductive mixture to start the rehydration of the osteoinductive system. Following the hydration, a pliable viscous putty is formed which is then applied to the operation site. The hydration can also be carried out by using the patient's own blood. The current system allows for the preparation of the final hydrated DMB* up to 10 cc—in practice the four sizes of 1 cc, 2 cc, 4 cc and 8 cc are routinely produced.

Clinical Trials

Prior to the general use of the newly developed osteoinductive system, detailed clinical trials were carried out by the Department of Orthopaedics at the University of Pretoria, following the formal approval by the Ethics Committee of the University.

Results and Discussion

Influence of Radiation Crosslinking on Bone Strength

The effect of the radiation crosslinking on the strength of the newly formed bone is given in Fig. 2. It was found that the radiation-crosslinked demineralised bone (DBM*) is more effective in initiating new bone throughout the time span of healing of the bone, and after three weeks the newly formed bone was more than three times *stronger* than the control as measured by compression testing.

Rate of New Bone Formation

Using the alkaline phosphatase (ALP) activity as an indicator for the osteoinductivity, it was found that the DBM* has a 30% higher ALP enzyme activity compared to that of the non-crosslinked DBM. As shown in Fig. 3 [13]. The relative gene expression of ALP activity revealed a gradual increase over time for both human (allograft) and animal (xenograft) as shown in Fig. 3 [13].

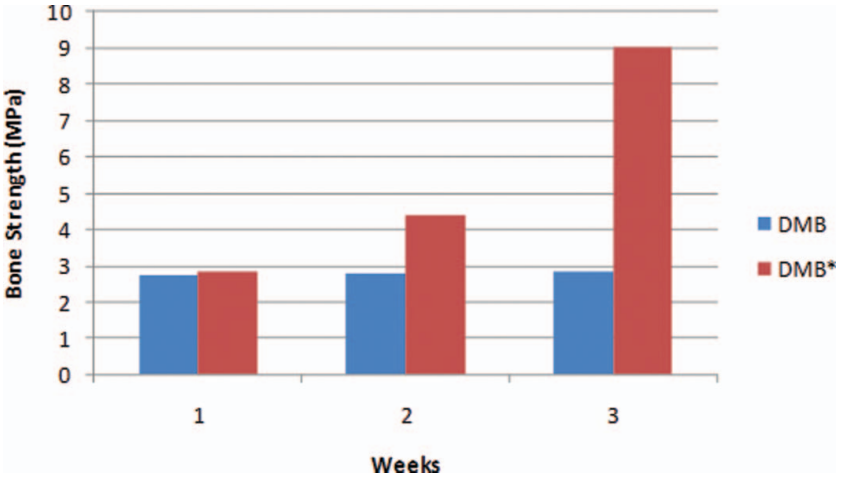

Figure 2. Comparison of inter-bony union strength (in MPa) at weeks 1 to 3 after implantation with non-crosslinked (DBM) and crosslinked (DBM*) demineralised bone.

Properties of Rehydrated DBM in Collagen*

Additional advantages of the rehydrated crosslinked demineralised bone in collagen as carrier, is its improved rate of rehydration, mechanical strength and relative insolubility, ensuring it does not wash away upon implantations. The more rapidly solidifying gel (*putty*) is retained more readily at the surgical site for the full dose to remain in place, despite bleeding in the area of the implant, an important consideration which has previously limited the use of demineralised bone in clinical settings.

Clinical Trials

Following two series of clinical trials carried out by the University of Pretoria and more than 1300 sets used in clinical practice over eighteen months in South Africa, the general market reaction has been so positive that it was decided to proceed with the commercialisation of

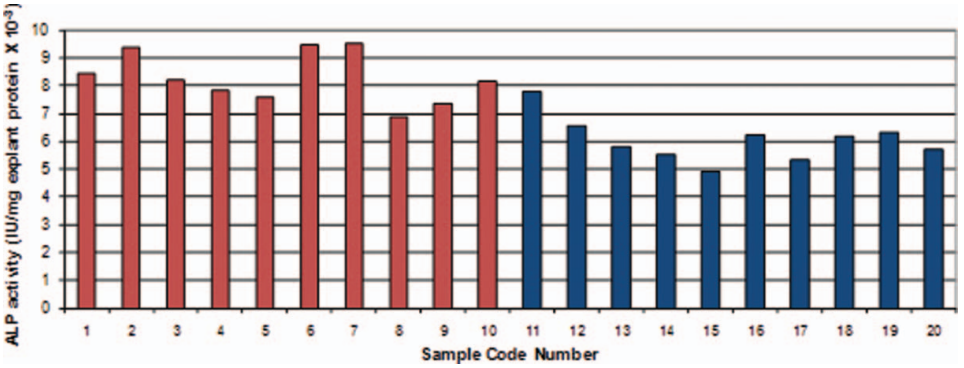

Figure 3. Alkaline phosphatase activity in crosslinked and non-crosslinked demineralised bone samples.

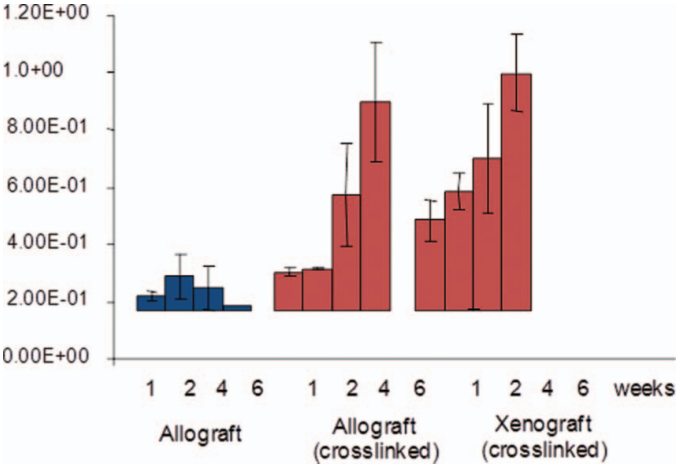

Figure 4. The relative gene expression of alkaline phosphatase activity for in vivo human and animal demineralised bone—crosslinked and non-crosslinked [13].

the product. To date more than 5 000 osteoinductive sets were used in South Africa—in oral, maxillofacial and orthopaedic surgery. The product is marketed in South Africa under the trademark Osteolink® by the National Tissue Bank of the University of Pretoria.

Conclusions

The following conclusions can be made on the radiation crosslinked demineralised bone matrix (DBM*) in collagen, and used in commercial clinical practice in South Africa since July 2004. The crosslinked DBM* shows a marked enhancement in osteogenic potential, providing a safe and easy-to-use demineralised allograft and xenograft as compared to non-crosslinked demineralised bone. The new bone growth resulting from the use of crosslinked DBM* shows an almost 300% increase in strength compared to that of non-crosslinked demineralised bone. A further advantage of the crosslinking of the DBM substrate is its increased mechanical strength and its relative insolubility, ensuring it does not wash away upon implantation, factors which have previously limited the use of DBM in surgical settings.

The formation of the putty or gel reconstituted from the dry crosslinked DBM* takes place much faster than the non-crosslinked equivalent—a property that is advantageous during implantation. The faster solidifying gels are also better retained at the implantation site, an important factor therapeutically under conditions of bleeding. This allows for the benefits of the full dose of DBM to be delivered to the surgical site.

The crosslinked dry DBM* is stored under inert nitrogen gas, eliminating oxidative degradation. This obviates the need to transport and store the product under refrigerated conditions, resulting in a shelf-life of at least five years. The product is reconstituted in fresh form in theatre directly before use.

The novel delivery system described herein has been developed specifically for this product and is superior to similar delivery systems presently available. The premixed lyophilised components are integrated in a single pack for ease of use and sterility is maintained throughout with no increased risk of contamination during reconstitution of the product.

The proprietary processing system allows for the osteoinductive mixture and water for re-hydration to be subjected to a terminal gamma radiation sterilisation process with the associated very high degree of sterility assurance and safety guaranteed to the patient. Clinical experience in South Africa has indicated that the product in its final packaged form is easy to use, and is both safe and clinically effective.

Acknowledgments

The authors wish to express their appreciation to the National Tissue Bank at the University of Pretoria for their involvement in this project, Dr. Malan de Villiers from Southern Medical for his valuable support and Ms. J. R. Tasker for her valuable contribution to the alkaline phosphatase analyses.

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