DECEMBER 2015, NO 5

Scripta-Ingenia

A admirável multiplicidade de Psi

S. Lladó, paintings and scultures, for a doctor's consulting rooms in Barcelona.

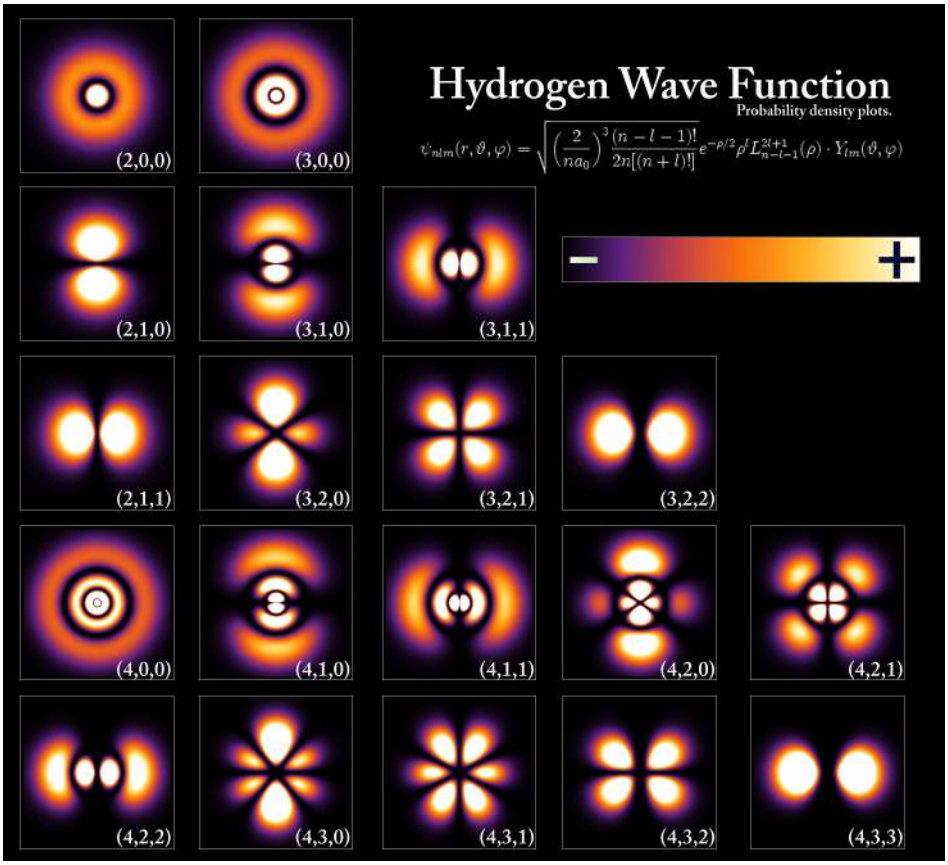
É admirável e não deixa de ser surpreendente a grande multiplicidade observada na utilização da letra Psi e nas suas relações intrinsecas que, se analizadas pelo prisma da verdadeira correlação entre significado e significância, nos abrem as portas a um vasto conhecimento humano que se vem acumulando ao longo de séculos ou milénios. A letra Psi constitui-se como a vigésima terceira letra (que corresponde também à penúltima) do alfabeto grego. A sua origem é incerta, tendo aparecido por volta do sétimo século anterior à nossa era e tem geralmente associada a conotação de Psyche, ou espírito humano, no sentido da existência de uma presença física imaterial. A sua utilização pode ser observada a dois níveis distintos: coincidência de acrónimos (1), ou significado intrínseco (2). A psicologia (de Psyche) será um exemplo da segunda ocorrência (2) ao passo que o índice de valores PSI-20 (Portuguese Stock Index) ou a unidade de medida PSI (pound per square inch) serão exemplos do primeiro tipo (1). No que respeita à psicologia, não deixa de ser interessante observar como os próprios psicólogos, e outros profissionais de saúde nessa àrea, se referem a si próprios pelo uso da letra que também adoptam como símbolo. Veja-se por exemplo o título do artigo de opinião de Maria Filomena Mónica, publicado no Jornal Público a 20 de Abril de 2001: *Os "psis": psiquiatras, psicanalistas e psicólogos*, no qual expõe a sua opinião sobre os cuidados a ter no recurso exagerado a consultas-psi. De facto, é muito comum ouvir entre esses profissionais de saúde a expressão *nós os psis* para se referirem a eles próprios. Um psi pode assim significar um psicólogo, um email PSI (para sua informção), ou uma libra por polegada quadrada que é uma unidade de pressão no sistema inglês. A pressão é a grandeza física que mede a força que se exerce por unidade de área, a psicologia é o instrumento utilizado para medir a distância entre cada um dos indivíduos de uma comunidade em relação aos seus valores médios, os quais são genericamente aceites por essa mesma comunidade. Um email psi é apenas uma forma neutra e impessoal de comunicar informação, por vezes utilizada pela dificuldade em determinar o nível apropriado de intimidade a manifestar na abertura e no fecho do email. E no entanto, existe também a possibilidade de se sentir psi. O sentir-se psi, em si, não contraria a possibilidade de uma existência material, apenas reflete a capacidade que é atribuída ao ser humano de, em certos momentos da sua vida, poder assumir que a sua existência não se manifesta apenas naquilo que pensa mas também se vê naquilo que sente. O Psi é um símbolo muito utilizado também na Matemática, na Física, na Química e na Engenharia. A título de exemplo, podemos observar que não terá sido por mero acaso que Erwin Schrödinger o tenha escolhido para representar a sua função de onda, expressa na equação que também tem o seu nome.

$$i\hbar\frac{\partial}{\partial t}\Psi = \hat{H}\Psi \quad \text{(Equação de Schrödinger)}$$

É notável como esta equação (ver página seguinte para mais pormenores sobre ela), apesar de não nos dizer de que é feita a matéria, e por isso não justificar a nossa existência corpórea, faz previsões correctas sobre o seu comportamento, e sobre a sua capacidade de se sentir psi, ou seja, de poder estar aqui e ali.

A Scripta-Ingenia assume-se como uma revista de divulgação científica tratando temas da ciência e da tecnologia, cobrindo todas as áreas do saber no domínio das ciências exactas ou aplicadas. Interessa-se ainda por artigos de opinião, sobre tópicos científicos ou não, desde que escritos por autores na área das ciências e da engenharia, e que reflitam as suas opiniões enquanto membros dessa comunidade.

Director and Chief Editor — Nelson Martins-Ferreira
CDRSP-ESTG, IPLeiria



Solution to Schrödinger's equation for the hydrogen atom at different energy levels. The brighter areas represent a higher probability of finding an electron.

A equação de onda de Schrödinger (ver página anterior) determina a evolução da função de onda, Ψ, ao longo do tempo. Na equação, o símbolo i representa a unidade imaginária, $\hbar$ a constante de Planck dividida por 2π, e $\hat{H}$ é o operador Hamiltoniano que caracteriza a energia total de Ψ; este operador depende do sistema a analizar e não é único. Por exemplo, no átomo de Hidrogéneo admite-se que

$$\hat{H} = -\frac{\hbar^2}{2\mu}\nabla^2 - \frac{Ze^2}{4\pi\epsilon_0 r}$$

é obtido pela energia cinética radial (primeiro termo) combinada com a força de atracção de Coulomb, entre o electrão e o protão no átomo (segundo termo). As suas soluções podem ser observadas na figura acima para várias combinações do triplo (n, l, m) que especifica, respectivamente, os números quânticos principal, de momento angular, e magnético. Obtemos assim, pelo princípio da superposição, uma previsão para o comportamento do átomo. São as consequências da previsão desse comportamento que são objecto de estupefacção na medida em que sugerem que a matéria nada mais é do que uma espécie de psyche que tem a possibilidade de estar em toda a parte, por um lado, e ao mesmo tempo em parte nenhuma, pelo menos até ao ponto em que seja feita uma observação. Tal é a veracidade da equação...

Silk Hydrogels for Tissue Engineering

by KHOON S. LIM(*) & TIM B. F. WOODFIELD

Christchurch Regenerative Medicine and Tissue Engineering (CReaTE) Group, Department of Orthopaedic Surgery and Musculoskeletal Medicine, University of Otago Christchurch, Christchurch 8011, New Zealand.
(*) Corresponding author Email: khoon.lim@otago.ac.nz

Abstract Silk hydrogels have been highlighted in the past decade as potential matrices for tissue engineering and regenerative medicine applications. In this mini review, the biological attributes of silk proteins, as well as methods reported in literature to fabricate silk hydrogels will be discussed.

1 Introduction

The field of tissue engineering (TE) which aims to repair, regenerate or replace damaged tissue has been extensively researched in the past decade as an alternative to organ transplantation. The general approach has been centred on combining cells, growth factors and tissue engineering matrices, with the goal of engineering functional tissues in vitro, which can then be implanted into the body. Hydrogels, which are highly hydrated polymeric network have been highlighted as potential tissue engineering matrices, due to their structural similarity to the native extracellular matrix [1, 2]. Several materials ranging from synthetic to natural polymers, have been fabricated into hydrogels, and shown to support cellular growth and differentiation [2]. In particular, silk proteins, which have been traditionally used in biomedical applications as sutures and drug delivery systems, have also been translated into tissue engineering matrices in the form of hydrogels. This review will focus on the main attributes of silk proteins as biomaterials, as well as methods to fabricate silk hydrogels for tissue engineering applications.

2 Hydrogels as tissue engineering matrices

Hydrogels are defined as hydrophilic polymeric networks which are capable of absorbing water ranging from ten to a thousand times their dry weight [3]. They are structurally similar to the native extracellular matrix (ECM) in their hydrated state, which enables facilitation of nutrient diffusion and waste removal through the TE matrix. In an ideal scenario, when cells are encapsulated within a hydrogel, the hydrogel should be able to support cell remodelling and ECM secretion, which results in a functional engineered tissue that can be transplanted (Figure 1). Hydrogels can be classified based on the forces between the crosslinked networks (physical or covalent), or the nature of the polymer used for fabrication (synthetic or natural) [2]. Physical hydrogels have either molecular entanglements, ionic bonding, or hydrogen bonding holding the network together that can be reversible, while covalent gels are hydrogels with covalently crosslinked networks that are permanent [3]. These gels can be fabricated from a range of synthetic or natural polymers.

In general, synthetic hydrogels have less batch-to-batch variability with good chemical and mechanical stability. Current synthetic polymeric hydrogel systems reported in literature include poly(vinyl alcohol) (PVA), poly(ethylene oxide) (PEO), poly(ethylene glycol) (PEG), poly(N-isopropylacrylamide) (NIPAAm), and poly(propylene furmarate) (PPF) [1, 2, 4-8]. However, although the reported synthetic hydrogels have good physico-mechanical properties, their application as scaffolds for TE remain limited due to the lack of biological sequences available to facilitate cellular functions. Moreover, most of these polymers are hydrophilic and resist cell attachment [9, 10].

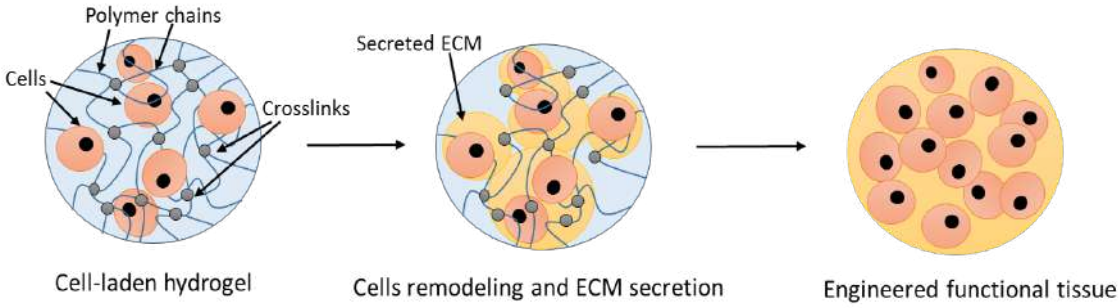


Figure 1: Schematic of tissue formation in cell-laden hydrogels.

On the other hand, hydrogels fabricated from naturally derived polymers, such as chitosan, collagen, gelatin, hyaluronic acid, fibrinogen and fibrin, are known to have good bio-functionality to support cellular growth, proliferation and differentiation [11-16]. However, these natural hydrogels are generally weak with limited mechanical stability as TE matrices. Therefore, there exists a need to engineer TE scaffolds that have the advantages of both synthetic and natural polymers, with good physico-mechanical properties and also with the ability to support cellular function. In recent years, hydrogels fabricated from silk proteins have been identified as potential candidates to meet these criteria.

3 Silk proteins

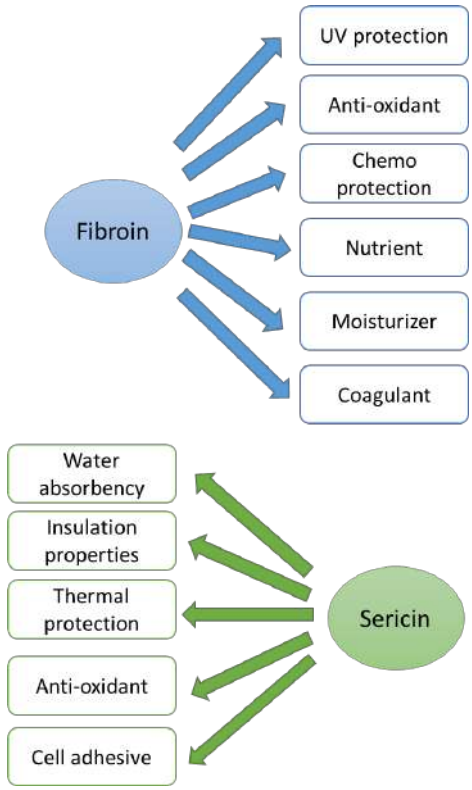


Figure 2: Biological attributes of silk fibroin and sericin [18].

Silk is a combination of fibrous proteins synthesised in specialised epithelial cells that line glands in silkworms, and has been successfully used as a suture material for centuries with great potential in biomaterial applications [17]. Before converted to silk fibers, silk proteins are synthesized by silk gland cells and stored in the lumen of the silk glands [18]. The usage of silk fibers is advantageous in the biomaterial aspect as it has properties that can rival synthetic polymers but requires less harsh processing conditions [18]. Silks have impressive mechanical properties, environmental stability, biocompatibility, controlled proteolytic biodegradability, morphologic flexibility and the ability for amino acid side change modification to immobilize growth factor [17].

The two major components in silk is fibroin and sericin, where fibroin is normally coated with sericin in the cocoons [18]. The biological attributes of these two silk proteins are listed in Figure 2. Sericin is secreted from the middle silk gland of a mature silkworm larva and acts as the glue that keeps fibroin together [19, 20]. Sericin produced by the most commonly researched domesticated type, Bombyx mori,(B. mori) consists of peptides with 3 major fractions of 150, 250, and 400 kDa [21]. This protein also exists in two kinds of conformation, random coils or β-sheets. [18].

On the other hand, fibroin is the major structural protein of silk which is secreted from the posterior silk gland [19]. Vepari et al reported that B. mori fibroin fibers are about 10-25 μm in diameter and contain a light protein chain (L-chain) with molecular weight of approximately 26 kDa, and heavy protein chain (H-chain) of approximately 390 kDa, where both L- and H-chain are linked by a disulphide bond [17]. Similarly to sericin, fibroin also exists in random coils and β-sheets conformation. By heating, stretching or immersing fibroin in a polar solvent, the protein conformation undergoes transition from random coil to β-sheet (Figure 3). This β-sheet transformation also corresponds to higher mechanical durability, where higher amount of β-sheet formation corresponds to higher mechanical strength [22].

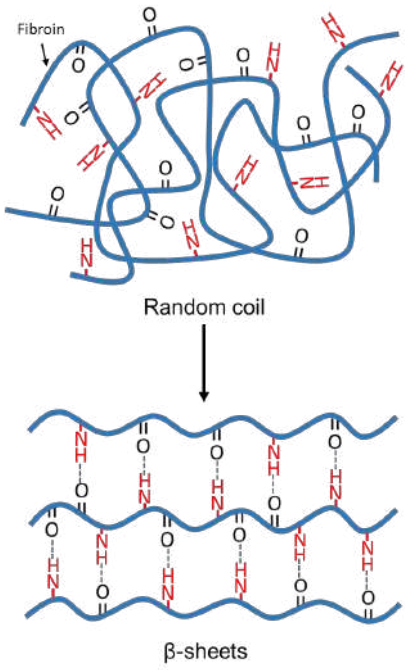


Figure 3: Random coil to β-sheet transformation of silk fibroin.

4 Silk hydrogels: Fabrication techniques and applications

For the past decades, it has been thought that the sericin fraction of silk causes unwanted inflammatory responses and hence has not been the major focus of biomaterial research. Sericin, which is the glue component of the silk cocoons are normally isolated using heat in basic conditions. These isolation conditions have been shown to cause denaturation and degradation of the protein, which subsequently affects the mechanical properties of the resultant hydrogels. In order to prevent heat denaturation during sericin isolation, Teramoto et al. researched genetically modified silkworms (Sericin-Hope) whose cocoons contain 99% sericin. As these Sericin-Hope cocoons contain almost no fibroin, the heat treatment was not required to separate the sericin from fibroin [23, 24]. However, although these hydrogels were shown to have significantly higher elastic modulus than the conventionally used domesticated silkworms, their mechanical properties were still not on par with fibroin hydrogels [23, 24].

Fibroin, the fibrous part of the silkworm cocoon, has been characterised to have abundance of hydrophobic amino acid groups such as glycine, sericin and alanine, which are capable to form physical crosslinks without addition of any chemical crosslinkers [25]. This approach is beneficial as concerns associated with toxicity of chemical crosslinkers are eliminated. However, fibroin hydrogels formed by this self-assembly approach require a long crosslinking time that can take up to days. Kim et al. showed that silk fibroin solution (2% w/v, pH 6.4 - 6.8, 37 °C) required 30 days for complete gelation [20]. These large hydrophobic domains of fibroin also allow its gelation time to be tailored by a number of factors such as temperature, ionic concentration, pH and salt concentration [26]. The different factors used to control fibroin gelation are:

pH changes pH closer to fibroin isoelectric point accelerate gelation [27-29]

Temperature changes Fibroin solution crosslinks faster at higher temperature. The resultant hydrogels are also mechanically stiffer [20, 27, 30]

Freeze-thawing Porosity of fabricated fibroin hydrogels depend on the number of freeze-thaw cycles [31-33]

For example, Ayub et al. showed that decreasing the pH of the fibroin solution to 3-4 successfully facilitated gel formation within two days [34]. It was hypothesised that the lower pH initiated protonation of the carboxyl groups, which subsequently reduce repulsion between the fibroin polymer chains and led to formation of crosslinks [29, 35]. It has also been showed that the fibroin protein structures are converted from random coils to β-sheets during the crosslinking process [34]. As the physico-mechanical properties of the fibroin gels are directly related to the amount of β-sheets formed, many researchers have focused on different methods to induce β-sheet transition [20, 27, 36, 37]. The compressive modulus of these fibroin gels fabricated using different methods can vary from 60 kPa to 7 GPa [26]. Chemical crosslinking of fibroin solution using different solvents and chemicals has also been studied to fabricate fibroin hydrogels. Although these gels are normally mechanically stronger and require shorter gelation time, the chemicals or conditions used are normally quite harsh for cells in terms of TE applications. For example, typical chemical crosslinkers include glycerol, sodium dodecyl sulfate and sodium N-lauroyl sarcosinate that are not cyto-compatible to cells at high concentrations [35, 38-40].

Fibroin hydrogels have gained huge popularity as TE matrices in the past decade for various applications such as bone engineering, cartilage engineering, nerve engineering, immunoisolation of cells, drug delivery and injectable void fillers. For example, mesenchymal stromal cells seeded into macroporous fibroin hydrogels were able to proliferate into osteoblasts and promote bone formation [41, 42]. Similarly. Fini et al. reported that the presence of fibroin gels in a cranial defect promoted bone healing by increasing the rate of osteoblasts proliferation and differentiation [43]. The rate of healing was significantly better than the FDA approved synthetic polymer, poly(lactide-co-glycolic acid) (PLGA) [41]. Fibroin gels have also been used to repair peripheral nerve injuries, where conduits coated with fibroin gels and immobilised with nerve growth factors successfully promoted nerve regeneration of a 14 mm rat sciatic nerve injury [44]. These hydrogels have good chemical and mechanical stability in vivo post implantation, which can be used as void fillers for surgical reconstruction and soft tissue augmentation [45].

All these examples highlighted the potential of fibroin hydrogels as tissue engineering matrices, mainly due to its inherent biocompatibility and biofunctionality properties, as well as tailorable physico-mechanical properties.

5 Conclusion and future outlooks

In conclusion, this review has outlined the main attributes of the two silk proteins, sericin and fibroin, mainly focusing on methods to fabricate them into hydrogels and their applications. Although these hydrogels have shown great potential as TE matrices, there is still lack of data on the in vivo performance of these materials to demonstrate that they are safe for clinical use. As the TE field is trending towards bridging the gap between in vitro and in vivo studies, future experiments should focus on evaluating the in vivo degradation, functionality and mechanical properties of the silk hydrogels, to confirm that these gels meet the clinical requirements.

References

1. Lim, K.S., et al., Promoting Cell Survival and Proliferation in Degradable Poly(vinyl alcohol)–Tyramine Hydrogels. Macromolecular Bioscience, 2015. 10(15): p. 1423-1432.

2. Nicodemus, G. and S.J. Bryant, Cell encapsulation in biodegradable hydrogels for tissue engineering applications. Tissue Engineering, Part B, 2008. 14(2): p. 149-65.

3. Rosiak, J.M. and F. Yoshii, Hydrogels and their medical applications. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms, 1999. 151(1-4): p. 56-64.

4. Lim, K.S., et al., Covalent incorporation of non-chemically modified gelatin into degradable PVA-tyramine hydrogels. Biomaterials, 2013. 34(29): p. 7097-7105.

5. Dadsetan, M., et al., Characterization of photocross-linked oli[poly(ethylene glycol) fumarate] hydrogels for cartilage tissue engineering. Biomacromolecules, 2007. 8(5): p. 1702-1709.

6. Ali, S., et al., Immobilization of Cell-Adhesive Laminin Peptides in Degradable PEGDA Hydrogels Influences Endothelial Cell Tubulogenesis. Biores Open Access, 2013. 2(4): p. 241-9.

7. Benoit, D.S.W. and K.S. Anseth, Heparin functionalized PEG gels that modulate protein adsorption for hMSC adhesion and differentiation. Acta Biomaterialia, 2005. 1(4): p. 461-470.

8. Adrus, N. and M. Ulbricht, Rheological studies on PNIPAAm hydrogel synthesis via in situ polymerization and on resulting viscoelastic properties. Reactive and Functional Polymers, 2013. 73(1): p. 141-148.

9. Chen, Y.m., et al., Synthetic hydrogels as scaffolds for manipulating endothelium cell behaviors. Chinese Journal of Polymer Science (English Edition), 2010: p. 1-19.

10. Chen, M.W., M. Gupta, and K. Cheung, Alginate-based microfluidic system for tumor spheroid formation and anticancer agent screening. Biomedical Microdevices, 2010. 12(4): p. 647-654.

11. Bhattarai, N., J. Gunn, and M. Zhang, Chitosan-based hydrogels for controlled, localized drug delivery. Advanced Drug Delivery Reviews, 2010. 62(1): p. 83-99.

12. Benton, J.A., et al., Photocrosslinking of gelatin macromers to synthesize porous hydrogels that promote valvular interstitial cell function. Tissue Eng Part A, 2009. 15(11): p. 3221-30.

13. Masters, K.S., et al., Crosslinked hyaluronan scaffolds as a biologically active carrier for valvular interstitial cells. Biomaterials, 2005. 26(15): p. 2517-2525.

14. Park, S.H., et al., Tissue-engineered cartilage using fibrin/hyaluronan composite gel and its in vivo implantation. Artificial organs, 2005. 29(10): p. 838-845.

15. Elvin, C.M., et al., Evaluation of photo-crosslinked fibrinogen as a rapid and strong tissue adhesive. J Biomed Mater Res A, 2010. 93(2): p. 687-695.

16. Janmey, P.A., J.P. Winer, and J.W. Weisel, Fibrin gels and their clinical and bioengineering applications. Journal of The Royal Society Interface, 2009. 6(30): p. 1-10.

17. Vepari, C. and D.L. Kaplan, Silk as a biomaterial. Progress in Polymer Science, 2007. 32(8-9): p. 991-1007.

18. Mondal, M., K. Trivedy, and S. Nirmal Kumar, The silk proteins, sericin and fibroin in silkworm, Bombyx mori Linn., - a review. Caspian Journal of Environmental Sciences, 2007. 5(2): p. 63-76.

19. Dash, R., et al., Purification and biochemical characterization of a 70 kDa sericin from tropical tasar silkworm, Antheraea mylitta. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 2007. 147(1): p. 129-134.

20. Kim, U.J., et al., Structure and Properties of Silk Hydrogels. Biomacromolecules, 2004. 5(3): p. 786-792.

21. Dash, R., S. Mukherjee, and S.C. Kundu, Isolation, purification and characterization of silk protein sericin from cocoon peduncles of tropical tasar silkworm, Antheraea mylitta. International Journal of Biological Macromolecules, 2006. 38(3): p. 255-258.

22. Rujiravanit, R., et al., Preparation of Crosslinked Chitosan/Silk Fibroin Blend Films for Drug Delivery System. Macromolecular Bioscience, 2003. 3(10): p. 604-611.

23. Teramoto, H., et al., Role of Hydroxyl Side Chains in Bombyx mori Silk Sericin in Stabilizing Its Solid Structure. Macromolecules, 2007. 40(5): p. 1562-1569.

24. Teramoto, H., K. Nakajima, and C. Takabayashi, Preparation of elastic silk sericin hydrogel. Bioscience, Biotechnology, and Biochemistry, 2005. 69(4): p. 845 - 847.

25. Fournier, A., Quantitative data on the Bombyx mori L. silkworm: a review. Biochimie, 1979. 61(2): p. 283-320.

26. Kapoor, S. and S.C. Kundu, Silk protein-based hydrogels: Promising advanced materials for biomedical applications. Acta biomaterialia, 2015.

27. Motta, A., et al., Fibroin hydrogels for biomedical applications: preparation, characterization and in vitro cell culture studies. Journal of Biomaterials Science, Polymer Edition, 2004. 15: p. 851-864.

28. Fang, J.-Y., et al., Characterization and evaluation of silk protein hydrogels for drug delivery. Chemical and pharmaceutical bulletin, 2006. 54(2): p. 156-162.

29. Zhou, P., et al., Effects of pH and calcium ions on the conformational transitions in silk fibroin using 2D Raman correlation spectroscopy and 13C solid-state NMR. Biochemistry, 2004. 43(35): p. 11302-11311.

30. Ribeiro, M., et al., The role of dialysis and freezing on structural conformation, thermal properties and morphology of silk fibroin hydrogels. Biomatter, 2014. 4(1): p. e28536.

31. Min, S., et al., Preparation and characterization of crosslinked porous silk fibroin gel. Sekiyu Gakkaishi,

1998. 54(2): p. 85-92.

32. Morita, Y., et al., Visco-elastic properties of cartilage tissue regenerated with fibroin sponge. Bio-medical materials and engineering, 2002. 12(3): p. 291-298.

33. Guziewicz, N., et al., Lyophilized silk fibroin hydrogels for the sustained local delivery of therapeutic monoclonal antibodies. Biomaterials, 2011. 32(10): p. 2642-2650.

34. Ayub, Z.H., M. Arai, and K. Hirabayashi, Mechanism of the gelation of fibroin solution. Bioscience, biotechnology, and biochemistry, 1993. 57(11): p. 1910-1912.

35. Matsumoto, A., et al., Mechanisms of silk fibroin sol-gel transitions. The Journal of Physical Chemistry B, 2006. 110(43): p. 21630-21638.

36. Motta, A., et al., Stabilization of Bombyx mori silk fibroin/sericin films by crosslinking with PEG-DE 600 and genipin. Journal of Bioactive and Compatible Polymers, 2011. 26(2): p. 130-143.

37. Minoura, N., et al., Attachment and growth of cultured fibroblast cells on silk protein matrices. Journal of Biomedical Materials Research, 1995. 29: p. 1215-1221.

38. Wu, X., et al., Sodium dodecyl sulfate-induced rapid gelation of silk fibroin. Acta Biomaterialia, 2012. 8(6): p. 2185-2192.

39. Zhang, H., et al., Preparation and characterization of a novel spongy hydrogel from aqueous Bombyx mori sericin. e-Polymers, 2008(066).

40. Zhang, F., et al., Potential of biocompatible regenerated silk fibroin/sodium N-lauroyl sarcosinate hydrogels. Journal of Biomaterials Science, Polymer Edition, 2015. 26(12): p. 780-795.

41. Meinel, L., et al., Silk implants for the healing of critical size bone defects. Bone, 2005. 37(5): p. 688-698.

42. Kim, H.J., et al., Influence of macroporous protein scaffolds on bone tissue engineering from bone marrow stem cells. Biomaterials, 2005. 26(21): p. 4442-4452.

43. Fini, M., et al., The healing of confined critical size cancellous defects in the presence of silk fibroin hydrogel. Biomaterials, 2005. 26(17): p. 3527-3536.

44. Tang, S., et al., The effects of gradients of nerve growth factor immobilized PCLA scaffolds on neurite outgrowth in vitro and peripheral nerve regeneration in rats. Biomaterials, 2013. 34(29): p. 7086-7096.

45. Etienne, O., et al., Soft tissue augmentation using silk gels: an in vitro and in vivo study. Journal of periodontology, 2009. 80(11): p. 1852-1858.

A Fern

Organismos Geneticamente Modificados, Qual o limite?

by Ana Rita Fonseca

Centre for Rapid and Sustainable Product Development
Polytechnic Institute of Leiria
E-mail: ana.r.fonseca@ipleiria.pt

Este trabalho resulta de uma pesquisa bibliográfica, reflectindo desta forma a preocupação da autora sobre um assunto que poderá afectar a Europa, no decorrer da aprovação do tratado trasntlântico TTIP.

1 Introdução

Na iminência de aprovação do tratado transatlântico (TTIP) entre a EU e os EUA, um acordo que visa promover o livre comércio, várias são as medidas a adotar pelos estados membro, nomeadamente a entrada de alimentos transgénicos nas prateleiras dos supermercados (1). A utilização de organismos geneticamente modificados (OGMs) sempre foi uma questão muito controversa.

Apesar de numerosos estudos científicos e garantias da Food and Drug Administration (FDA) de que os alimentos GMs são seguros e nutritivos, um número de indivíduos e grupos de consumidores ainda não foi convencido, existindo uma forte crença na campanha de desinformação, e de conflitos de interesse envolvendo a indústria multimilionária da biotecnologia (2, 3, 4). Considerando no entanto os altos riscos financeiros envolvidos, são levantadas preocupações sobre a influência que os conflitos de interesse poderão ter na publicação artigos científicos sobre o risco para a saúde ou o valor nutricional dos produtos alimentares geneticamente modificados (3).Coloca-se então a seguinte questão: mas afinal o que são OGMs? Para que servem? Quais as vantagens e desvantagens?

OGM significa *organismo geneticamente modificado*. São organismos cujo material genético foi alterado artificialmente para lhes oferecer uma nova propriedade, como a resistência a uma doença, aos insectos, à seca ou para aumentar a produtividade de uma colheita. A transformação do genoma pode ocorrer dentro da própria espécie, ou envolver genes de espécies diferentes (organismos transgénicos), conferindo propriedades que o ser vivo anteriormente não possuía (6).

De facto, o ser humano vem manipulando animais e cruzando plantas desde o final da idade da pedra. Este processo chamado por Darwin de seleção artificial, explica a origem da diversidade de raças, de animais, plantas, e frutas, a partir de critérios seletivos humanos, de cruzamento seletivo de animais, plantas e outros seres vivos, com o objetivo de selecionar características vantajosas que tendem a ter um efeito maior na geração seguinte.

Existem inúmeras vantagens provenientes da utilização de OGM, tanto ao nível da saúde como do ambiente (6):

1. O alimento pode ser enriquecido com um componente nutricional essencial, como por exemplo, o arroz geneticamente modificado que produz vitamina A, essencial em países em vias de desenvolvimento.

2. É possível obter alimentos mais baratos devido à redução dos prejuízos, pois pode-se obter plantas resistentes a insetos, pragas, a herbicidas, a metais tóxicos do solo, a fungos, ao amadurecimento precoce, entre outros.

3. Existência de culturas de OGMs em ambientes hostis, no sentido de criar novas zonas de cultivo, evitando que se desgastem tanto outras.

No entanto, a mãe natureza dita as regras, e aquilo que o homem manipula e pretende controlar, contrariar, tem as suas desvantagens (6) e indiscutivelmente trará as suas consequências:

1. Impactos irreversíveis sobre a biodiversidade (6). As culturas geneticamente modificadas podem ter uma vantagem competitiva alterando os ecossistemas.

2. O lugar em que o gene é inserido não pode ser controlado completamente, o que pode causar resultados inesperados uma vez que outros genes podem ser afetados.

3. Não se sabe até que ponto os alimentos geneticamente modificados (GMs) afetam a saúde humana. A alimentação à base de OGMs é muito recente para poder garantir que não surjam problemas no futuro, visto que mesmo pequenas alterações podem produzir grande impacto ao longo de gerações.

4. Efeitos colaterais que não podem ser previstos.

De uma forma muito sucinta e simplista, inicialmente existiam 3 objetivos primordiais: a) acabar com a fome, b) tornar a agricultura mais ecológica e limpa, c) simplificar o processo produtivo. De facto só se verificou o terceiro ponto, na qual o produtor não tem de se preocupar com as ervas daninhas, visto as suas culturas serem resistentes aos herbicidas (5).

O facto é que a Natureza é utilizada como um laboratório, cujos efeitos a longo prazo não são possíveis de determinar. É sobretudo esta a razão pela qual esta a introdução de OGMs na alimentação, suscita tanta desconfiança na sociedade e pela qual garantias de segurança tão severas quanto possível são tão insistentemente exigidas, nomeadamente pela UE. Como tal, ainda serão necessários muitos estudos para avaliar o impacto que os OGM podem vir a ter no futuro (6). Atualmente, deparamo-nos com duas situações:

- Facto 1: Variedades de alimentos (milho, soja, algodão...) são geneticamente modificados para produzir uma proteína pesticida chamada toxina Bt (do Bacillus thuringiensis) (7), com o intuito de conferir maior resistência à planta.

- Facto 2: Os transgenes de plantações geneticamente modificadas "ingeridos" são transferidos para a flora intestinal, chegando mesmo a ser encontrados no sangue, e órgãos internos (20).

Agora, juntando os dois factos acima a um terceiro: Se o gene que cria a toxina Bt for transferido para as bactérias de nosso sistema digestivo, a nossa flora intestinal pode ser transformada numa fábrica viva de pesticida. O problema acima referido poderá ser a causa, para os inúmeros problemas apontados às culturas de OGMs, tanto nos seres vivos como no ambiente.

2 Implicações nos Seres Vivos

Inúmeros estudos em animais, detetaram riscos graves de saúde, bem como casos específicos de doença associados ao consumo de alimentos transgénicos; essas doenças vão desde a infertilidade, alterações nos níveis de insulina, doenças do sangue, lesões ao nível dos intestinos, morte fetal, tumores, e morte precoce (8-13).

No entanto, após duas décadas da comercialização de OGMs, não foram realizados testes em seres humanos, com seria de esperar. Num artigo publicado na revista Nature Biotecnology, Emily Waltz (15) levanta a seguinte questão: "serão as táticas prepotentes da indústria agrícola (...) que impedem a investigação pública e minam a aceitação pública do cultivo dos transgênicos?". No mesmo artigo, 26 cientistas do setor público queixaram-se à EPA (Environmental Protection Agency) de que a indústria biotecnológica está a limitar os seus direitos de estudar as culturas transgénicas comercializadas, afirmando que "Nenhuma pesquisa verdadeiramente independente pode ser realizada legalmente, no que toca a algumas questões críticas envolvendo estas culturas [por causa das restrições impostas]"; a revista Scientific American concordou (16) afirmando: "as empresas de biotecnologia têm o poder de veto sobre o trabalho de pesquisadores públicos". Em 2011, a Science Direct publicou um estudo (3) que documentou a existência de conflito de interesses na investigação sobre os riscos para a saúde e avaliação nutricional dos produtos geneticamente modificados.

2.1 Toxina Bt envolvida em respostas imunológicas (nomeadamente alérgicas)

A proteína Bt, encontrada em variedades de milho, apresenta seções de aminoácidos idênticos a alérgenos conhecidos (36). Ela é usada por agricultores na forma de

spray e por isso foi considerada inofensiva para o ser humano, no entanto a Toxina Bt encontrada em alguns transgénicos é mais tóxica e milhares de vezes mais concentrada do que na forma de spray, sendo responsabilizada por inúmeras respostas imunitárias significativas em humanos e outros seres vivos (37-43), (45-48).

Inúmeros relatos revelam coerência entre as reações e sintomas relacionados com sprays de Bt e aqueles relatados por trabalhadores de algodão na Índia (39, 40, 44) e nas Filipinas (50, 51). De facto pastores relataram que 25% dos seus rebanhos que pastavam em plantações de algodão Bt na índia, morreram em 5-7 dias. Cerca de 10 mil ovelhas mortas na região manifestaram reações alérgicas (52).

As alergias à soja dispararam para 50% em Inglaterra, logo após a soja GM ter sido introduzida (25). Se a soja foi a causa, pode ser devido a vários motivos: i) Por um lado a proteína GM que torna a soja resistente ao herbicida Roundup Ready, pode ser considerada um alérgeno (25) se se considerar que a sequência dos seus aminoácidos é idêntica aos alérgenos conhecidos (26). Embora nunca tenha existido um estudo exaustivo das biomoléculas constituintes da soja GM, foram detetadas mudanças imprevisíveis no ADN (29), sendo possível que estas alterações estejam implicadas no aumento dos níveis de alergénios na soja, sobretudo quando cozinhada (27, 28, 30). Na verdade, os sintomas identificados como problemas de digestão, fadiga crónica, dores de cabeça, letargia, problemas de pele, incluindo acne e eczema, poderão estar relacionados com a exposição ao glifosato, o ingrediente-chave no herbicida Roundup Ready (25); ii)É também possível que o produto da decomposição do glifosato, AMPA, que se acumula na soja GM, possa contribuir para as alergias (33); iii) Finalmente, a redução das enzimas pancreáticas verificada, responsáveis por suprimir determinadas proteínas, podem promover reações alérgicas (34, 35).

2.2 ADN GM está a sobreviver à digestão, e ao ser absorvido a afetar a função celular

Estudos realizados em animais demonstraram que o ADN ingerido pode viajar pelo corpo (23, 24) e influenciar a função celular. Os transgenes de plantações geneticamente modificadas ingeridos por animais foram encontrados no sangue, músculo, fígado e rins (22). O único teste publicado sobre alimentação humana com comida transgênica verificou que o material genético inserido na soja transgênica foi transferido para o DNA do aparelho digestivo, nomeadamente no intestino (20).

2.3 Toxicidade e problemas reprodutivos

Existe evidência substancial de toxicidade e efeitos reprodutivos associados com os alimentos geneticamente modificados. A alimentação animal com soja GM, induziu espermatozóides alterados (56), mudanças significativas no desenvolvimento do embrião (57), morte fetal, entre outros (10, 58).

3 Riscos no meio Ambiente

As culturas de OGMs têm levado a um crescente uso de pesticidas. Na verdade, mais de 90% das modificações genéticas prendem-se com o aumento do uso de herbicidas e pesticidas, resultante da resistência que as ervas daninhas/infestantes desenvolveram. As empresas de biotecnologia começaram a modificar geneticamente plantas para tolerar herbicidas mais fortes, nomeadamente o ácido 2,4-D-diclorofenoxiacético (2,4-D), o ingrediente chave do “agente laranja” usado durante a guerra do Vietnam, conhecido por deixar sequelas terríveis na população daquele país e nos próprios soldados norte-americanos (17). De facto, o impacto dos pesticidas e a frequência com que estão a ser encontrados em águas subterrâneas, rios, solos, ar e até mesmo na chuva, em paralelo com as culturas geneticamente modificadas, estão a saturar o meio ambiente, traduzindo-se em repercussões e impactos diretos na saúde (18).

A indústria da biotecnologia alega que a grande maioria da população dos EUA alimenta-se de alimentos GMs, e ninguém ficou doente. No entanto a engenharia genética, aplicada às culturas alimentares, tem revelado que muitas premissas-chave de segurança estão erradas, importando ter em linha de conta que nunca existiu monitorização e que pode levar várias décadas até que seja possível identificar a implicação da alimentação/suplementação transgénica na saúde, tal como aconteceu nos anos 80, onde cerca de 100 americanos morreram e entre 5-10 mil ficaram doentes, devido a um suplemento alimentar transgénico, chamado L-tryptophan (7).

De facto, sem pesquisas e testes clínicos em humanos não podemos saber se os alimentos transgénicos são realmente responsáveis. Talvez um dia seja possível, de uma forma segura e previsível alterar culturas alimentares para o benefício da humanidade e do ambiente.

Bibliografia

1. Grupo de Portugal para análise crítica ao Acordo UE-EUA (TTIP), O que esconde a Parceria Transatlântica (ttip)?, acedido em 13 de Outubro de 2015.

2. Greenpeace Germany, Genetic Roulette: The Documented Health Risks of Genetically Engineered, Foods, Yes! Books, Fairfield, IA, 2007.

3. Diels, J. et al., Association of financial or professional conflict of interest to research outcomes on health risks or nutritional assessment studies of genetically modified products, Elsevier, Food Policy 36 (2011) 197–203.

4. http://academicsreview.org/reviewed-content/genetic-roulette/, acedido em 15 de Outubro

5. RTP play, Programa 1 - O 007 dos Transgénicos Cientistas portugueses lideram investigação europeia para criar um biossensor que deteta transgénicos nos alimentos, Disponível em http://www.rtp.pt/play/p2063/e209918/ponto-de-partida, acedido a 13 Outubro de 2015.

6. Living.Bio, OGM – Vantagens e Desvantagens, Fev 2009.

7. Greenpeace Brasil, Transgênicos são inseguros e têm que ser banidos, Nov 2007.

8. Finamore A, Roselli M, Britti S, et al., Intestinal and peripheral immune response to MON 810 maize ingestion in weaning and old mice, J Agric. Food Chem. 2008; 56(23):11533-11539.

9. Malatesta M, Boraldi F, Annovi G, et al., A long-term study on female mice fed on a genetically modified soybean:effects on liver ageing. Histochem Cell Biol. 2008; 130:967-977.

10. Velimirov A, Binter C, Zentek J., Biological effects of transgenic maize NK603xMON810 fed in long term reproduction studies in mice, Report-Federal Ministry of Health, Family and Youth. 2008.

11. Ewen S, Pustzai A., Effects of diets containing genetically modified potatoes expressing Galanthus nivalis lectin on rat small intestine. Lancet. 354:1353-1354.

12. Kilic A, Aday M., A three generational study with genetically modified Bt corn in rats: biochemical and histopathological investigation, Food Chem. Toxicol. 2008; 46(3):1164-1170.

13. Kroghsbo S, Madsen C, Poulsen M, et al., Immunotoxicological studies of genetically modified rice expression PHA-E lectin or Bt toxin in Wistar rats, Toxicology. 2008; 245:24-34.

14. Parlamento Europeu- atualidade, Seis coisas que deve saber sobre os organismos geneticamente modificados, Out 2015.

15. Emily, W., BATTLEFIELD- Papers suggesting that biotech crops might harm the environment attract a hail of abuse from other scientists. Emily Waltz asks if the critics fight fair, NATURE Vol 461, Sep 2009.

16. 8 Scientific American, Do Seed Companies Control GM Crop Research, Jul 2009.

17. http://www.consciencia.net/2005/mes/06/vietnaeua-impunidade.html, acedido em 28 Outubro 2015.

18. http://gmo-awareness.com/resources/glyphosate/, acedido em 29 Outubro.

19. Gillam, C., Pesticide use ramping up as GMO crop technology backfires: study, Reuters– Science, 2012.

20. Scientific Committee on Plants, Opinion of the Scientific Committee on Plants Regarding the Genetically Modified, Glufosinate-Tolerant Rape Notified by the Agrevo Company, Fev 1998.

21. Zhang, L. et al., Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA, Cell Research, (2012): 107–126

22. Foss, A.S. Growing fatter on a GM diet, Science Nordic, 2012.

23. Garber, L., GM wheat may permanently alter human genome, spark early death, Out 2012.

24. Sposák, S., et al., Complete Genes May Pass from Food to Human Blood, Pone Journal, 2013.

25. Townsend, M. Why soya is a hidden destroyer. Daily Express, Mar 12, 1999.

26. Kleter, G.A. & Peijnenburg, A.A.C.M. Screening of transgenic proteins expressed in transgenic food crops for the presence of short amino acid sequences identical to potential, IgE-binding linear epitopes of allergens. BMC Struct. Biol. 2 (2002): 8–19.

27. Padgette, S.R. et al. The composition of glyphosate-tolerant soybean seeds is equivalent to that of conventional soybeans. J. of Nutrition 126, no. 4 (1996).

28. Pusztai, A. & Bardocz, S. GMO in animal nutrition: potential benefits and risks. Ch. 17, Biology of Nutrition in Growing Animals (Elsevier, 2005).

29. Windels, P. et al. Characterisation of the roundup ready soybean insert. Eur. Food Res. Technol. 213 (2001): 107–112.

30. Rang, A. et al., Detection of RNA variants transcribed from the transgene in roundup ready soybean, Eur. Food Res. Technol. 220 (2005): 438–443.

31. 23. Benbrook, C., Genetically engineered crops and pesticide use in the United States: The First Nine Years,Oct 2004.

32. Cox, C., Herbicide fact sheet: glyphosate, J. of Pest. Reform 24, no. 4, 2004.

33. Duke, S.O. et al., Isoflavone, Glyphosate and aminomethylphosphonic acid levels in seeds of glyphosate-treated, glyphosateresistant soybean, J. Agric. Food Chem. 51 (2003): 340–344.

34. Sandermann, H. Plant biotechnology: ecological case studies on herbicide resistance. Trends in Plant Sci. 11, no. 7 (Jul 2006): 324–328.

35. 27. Malatesta, M. et al., Ultrastructural analysis of pancreatic acinar cells from mice fed on genetically modified soybean, J. of Anat. 201, no. 5 (Nov 2002): 409.

36. Malatesta, M. et al., Fine structural analyses of pancreatic acinar cell nuclei from mice fed on GM soybean, Eur. J. Histochem. 47 (2003): 385–388.

37. Gendel, The use of amino acid sequence alignments to assess potential allergenicity of proteins used in genetically modified foods, Advan. in Food and Nutrition Research 42 (1998): 45–62.

38. Dutton, A. et al., Uptake of Bt-toxin by herbivores feeding on transgenic maize and consequences for

the predator Chrysoperia carnea, Ecol. Entomology 27 (2002): 441–7.

39. Romeis, J., Dutton, A., & Bigler, F., Bacillus thuringiensis toxin (Cry1Ab) has no direct effect on larvae of the green lacewing Chrysoperla carnea (Stephens), J. of Insect Phys. 50, no. 2–3 (2004): 175–183.

40. Washington State Dept. of Health, Report of health surveillance activities: asian gypsy moth control program, Washington State Dept. of Health, Olympia, WA, 1993.

41. Green, M. et al., Public health implications of the microbial pesticide Bacillus thuringiensis: an epidemiological study, Amer. J. Public Health 80, no. 7 (1990): 848–852.

42. Noble, M.A., Riben, P.D., & Cook, G.J., Microbiological and epidemiological surveillance program to monitor the health effects of Foray 48B BTK spray, Ministry of Forests, Vancouver, B.C., Sept 30, 1992.

43. Swadener, C.,Bacillus thuringiensis, J. of Pest. Reform 14, no. 3 (Fall 1994).

44. Samples, J.R. & Buettner, H., Ocular infection caused by a biological insecticide, J. Infectious Dis. 148, no. 3 (1983): 614.

45. Gupta, A. et al., Impact of Bt cotton on farmers' health , Investigation Report, Oct–Dec 2005.

46. Vazquez et al. Intragastric and Intraperitoneal Administration of Cry1Ac protoxin from Bacillus thuringiensis induces systemic and mucosal antibody responses in mice. Life Sci. 64, no. 21 (1999): 1897–1912.

47. Vazquez et al., Characterization of the mucosal and systemic immune response induced by Cry1Ac protein from Bacillus thuringiensis HD 73 in mice, Brazilian J. of Med. and Biol. Research 33 (2000): 147–155.

48. Vazquez et al., Bacillus thuringiensis Cry1Ac protoxin is a potent systemic and mucosal adjuvant. Scandanavian J. of Immunology 49 (1999): 578–584.

49. Freese, B. The StarLink affair. Submission by Friends of the Earth to the FIFRA scientific advisory panel considering assessment of additional scientific information concerning StarLink corn (Jul 17–19, 2001).

50. Smith, J.M., Bt-maize (corn) during pollination, may trigger disease in people living near the cornfield, Press release, Feb 2004.

51. Ho, M., GM ban long overdue, dozens ill & five deaths in the Philippines, ISIS press release, Jun 2006.

52. Pradesh, A., Mortality in sheep flocks after grazing on Bt cotton fields—Warangal district, Apr 2006.

53. Seralini, G., Cellier, D., & Spiroux de Vendomois, J., New analysis of a rat feeding study with a genetically modified maize reveals signs of hepatorenal toxicity, J. archives of Env. Contam. and Toxicology (Springer, New York).

54. Strodthoff, H. & Then, C., Is GM maize responsible for deaths of cows in Hesse?,Greenpeace, Germany, Dec 2003.

55. Malatesta, M. et al., Ultrastructural morphometrical and immunocytochemical analyses of hepatocyte nuclei from mice fed on genetically modified soybean, Cell Struct. Funct. 27 (2002): 173–180.

56. Vecchio, L. et al., Ultrastructural analysis of testes from mice fed on genetically modified soybean, Eur. J. of Histochem. 48, no. 4 (Oct–Dec 2004):449–454.

57. Oliveri et al., Temporary depression of transcription in mouse pre-implantion embryos from mice fed on genetically modified soybean, 48th Symposium of the Society for Histochemistry, Lake Maggiore, Italy, (2006) 7–10.

58. Ermakova, I., Genetically modified soy leads to the decrease of weight and high mortality of rat pups of the first generation. Preliminary studies. Ecosinform 1 (2006): 4–9.

Wind mill, Pinhoa - Portugal

Orthogonal Perfect DFT Golay Codes

by J.M. LANZA-GUTIERREZ, M. FERREIRA, J.A. GOMEZ-PULIDO, S. PRIEM-MENDES, J.S. PEREIRA AND H.J.A. SILVA

J.M. Lanza-Gutierrez (*Technical University of Madrid, Centre of Industrial Electronics, Madrid, Spain*)

J.A. Gomez-Pulido (*University of Extremadura, Dep. of Technologies of Computers and Communications, Polytechnic School, Campus Universitario s/n, 10003 Caceres, Spain*)

M. Ferreira, J.S. Pereira and S. Priem-Mendes (*Polytechnic Institute of Leiria, School of Technology and Management, Leiria, Portugal*)

H.J.A. Silva and J.S. Pereira (*Instituto de Telecomunicações, Portugal*)

E-mail: jm.lanza@upm.es

Abstract Deciding about which coding sequences should be used is an important issue in many communication systems, such as those employing Code Division Multiple Access (CDMA). In this paper, we propose a cascading electronic codec based on a recursive algorithm for the generation of perfect sequences, derived from an Inverse Discrete Fourier Transform (IDFT) of Golay codes. The sequences generated have a low Peak-to-Average Power Ratio (PAPR), around 3 dB, which is the PAPR of a sine wave. The proposal is compared to the original Golay codes, verifying that our codec provides better crosscorrelation and autocorrelation results, crucial to gain immunity to multi-path interference.

1 Introduction

Many communication systems, such as those employing Code Division Multiple Access (CDMA), require appropriate coding sequences, which should have a perfect periodic autocorrelation and excellent crosscorrelation properties for synchronization or code detection in noisy environments. Some well-known orthogonal codes used for CDMA are Golay codes [1], Frank and Chu perfect sequences [2], and Gold codes [3] [4].

It is well-known that perfect sequences are complex sequences, having all out-of-phase periodic autocorrelation values equal to zero. Unfortunately, perfect bipolar sequences of length greater than 4 and perfect quadri-phase sequences of length greater than 16 are unknown [5].

In this paper, we propose an encoder of perfect sequences of length 2^N, with $N \in \mathbb{N}$, which are derived from an Inverse Discrete Fourier Transform (IDFT) of Golay codes. The codes obtained are complementary perfect sequences [6] with a low Peak-to-Average Power Ratio (PAPR). Because of their correlation properties they are immune to Multi-Path Interferences (MPI) [6]. These sequences are called Orthogonal Perfect DFT Golay (OPDG) codes [7].

2 OPDG generator

Figure 1 illustrates a simplified OPDG encoder, based on a discrete constant input signal A, generating two OPDG codes a_N and b_N. Those codes are then converted to analog signals by the Digital-to-Analog Converter (DAC) and sent through the physical transmission medium, which will contaminate them with noise. The new codes are received and converted to digital by the Analog-to-Digital Converter (ADC). Finally, the OPDG decoder recovers the original input signal A.

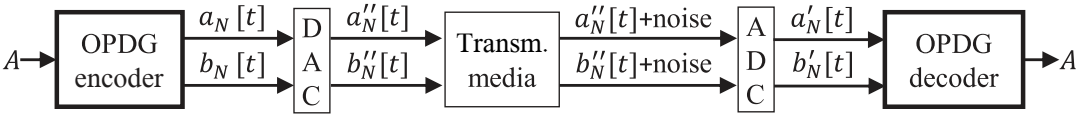


Figure 1: Generic OPDG application diagram.

The OPDG encoder (Figure 2, see next page) generates a pair of perfect orthogonal sequences, $a_N[t]$ and $b_N[t]$, of size $L = 2^N$. According to Figure 2, the encoder is composed of N cascading basic modules, each one with an adder, a subtractor, and a multiplier. Thus, given two complex input vectors $a_{n-1}[t]$ and $b_{n-1}[t]$ $n \in 1, \ldots, N$,

the basic module generates two new values $a_n[t]$ and $b_n[t]$ given by:

$$a_n[t] = a_{n-1}[t] + q \cdot W_L^{-t \cdot 2^{n-1}} \cdot b_{n-1}[t] \quad (1)$$

and

$$b_n[t] = a_{n-1}[t] - q \cdot W_L^{-t \cdot 2^{n-1}} \cdot b_{n-1}[t], \quad (2)$$

where $q = \pm 1$, t is the temporal displacement of the sequences, and with initial conditions $a_0[t] = A$ and $b_0[t] = A$, where A is a constant sequence of L discrete values, all of them assuming values 1 or -1. Let W_L be the twiddle factor

$$W_L = \exp\left(-j \cdot \frac{2\pi}{L}\right), \quad (3)$$

where j is $\sqrt{-1}$.

An important parameter of a signal in wireless communication systems is the Peak-to-Average Power Ratio. As it is well-known, this ratio affects the power amplifiers efficiency and cost. The PAPR is defined as the peak signal amplitude squared divided by the root mean square of the signal squared. It can be calculated (in dB) with

$$\text{PAPR} = 10 \cdot \log_{10}\left(\frac{{|x|_{peak}}^2}{{x_{rms}}^2}\right), \quad (4)$$

where x is the complex modulus of the OPDG code. Applying (4) to a_N and b_N gives results very similar to the sine wave PAPR: ≈ 3.01 dB. We have calculated the PAPR for OPDG sequences for $N \in 3, \ldots, 20$ and the result remains stable around 3 dB. The only exception is with $N = 4$, where the PAPR is even lower (2.3 dB).

The OPDG decoder (Figure 3) implements an autocorrelation function. If the input sequences are correct, i.e. $a'_N[t] = a_N[t]$ and $b'_N[t] = b_N[t]$, the decoder generates two outputs: an autocorrelation function proportional to the Dirac impulse $\delta(t)$, and a constant function proportional to the input signal A of the encoder. Otherwise, a null crosscorrelation function is obtained. The OPDG decoder is also composed of N basic modules. In each module, given two input vectors, $a'_n[t]$ and $b'_n[t]$, the module calculates two new values $a'_{n-1}[t]$ and $b'_{n-1}[t]$, as follows

$$a'_{n-1}[t] = q \cdot W_L^{-t \cdot 2^{n-1}} \cdot (a'_n[t] + b'_n[t]) \quad (5)$$

and

$$b'_{n-1}[t] = a'_n[t] - b'_n[t]. \quad (6)$$

The last block of Figure 3 implements the real part of a Fast Fourier Transform (FFT), providing a Dirac impulse of amplitude $A[q]^N 2^{2N+1}$ with a shift of $2^N - 1$.

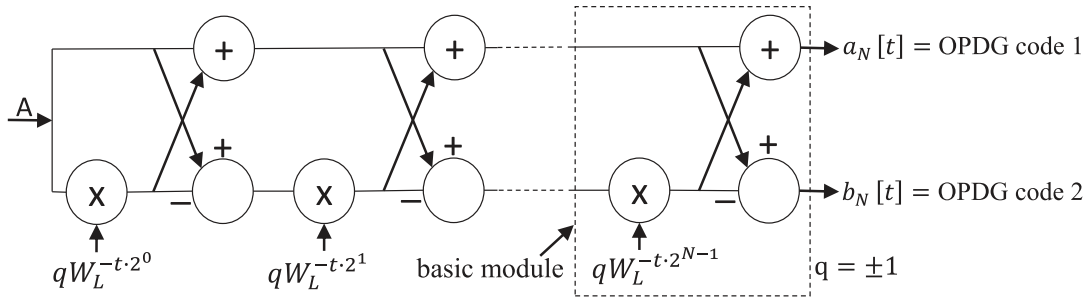


Figure 2: OPDG encoder.

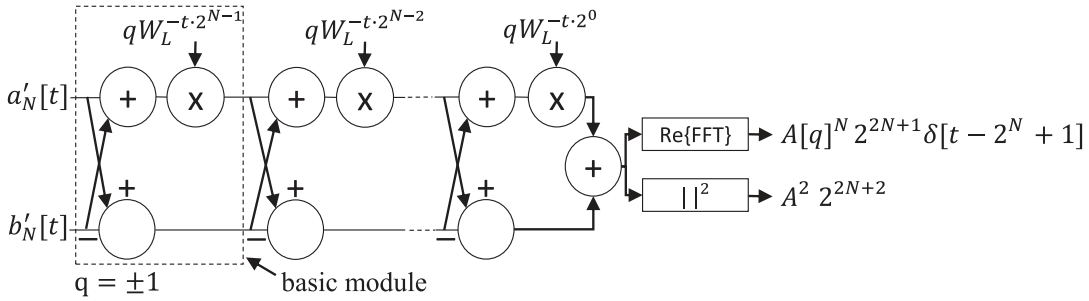


Figure 3: OPDG decoder.

3 Comparisons with other approaches

In this section, we compare our proposal to the well-known Golay codes. We generated a pair of bipolar sequences of length 2^N, where N equals 5, with the aim of analysing the behavior of the codes. We consider both autocorrelation and crosscorrelation functions.

Figure 4 (see following page) shows periodic autocorrelation functions for OPDG 1 and 2 [7], Golay 1 and 2 [1]. As shown, the OPDG codes provide a perfect periodic autocorrelation, while Golay codes have out-of-phase peaks. Since each OPDG code is complex valued, it can be split into real part $\text{Re}(OPDG1)$ and imaginary

part $\mathrm{Im}(OPDG2)$. These two sequences are complementary orthogonal sequences. The crosscorrelation properties of the generated codes are illustrated in Figure 5. As this plot shows, the crosscorrelation of $\mathrm{Re}(OPDG1)$ with $\mathrm{Im}(OPDG1)$, as well as the crosscorrelation of $\mathrm{Re}(OPDG2)$ with $\mathrm{Im}(OPDG2)$, is always zero, regardless of the phase and for any length (N). This does not happen with the crosscorrelation of Golay codes.

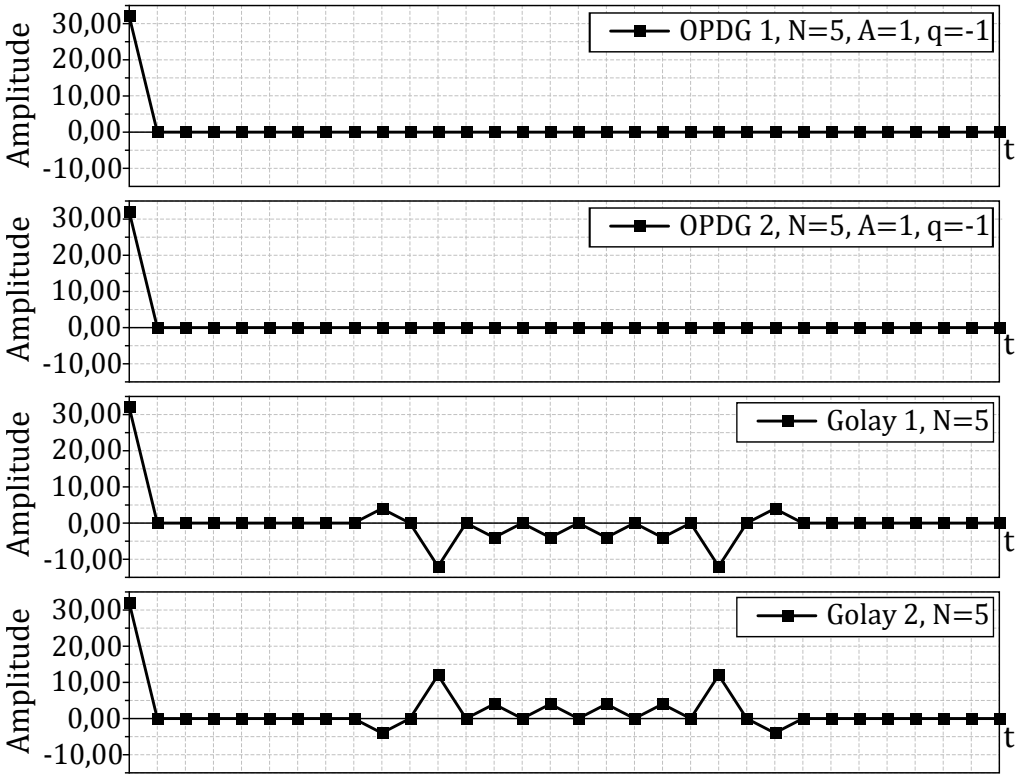


Figure 4: Periodic autocorrelation amplitude vs. code index t, for length 2^5.

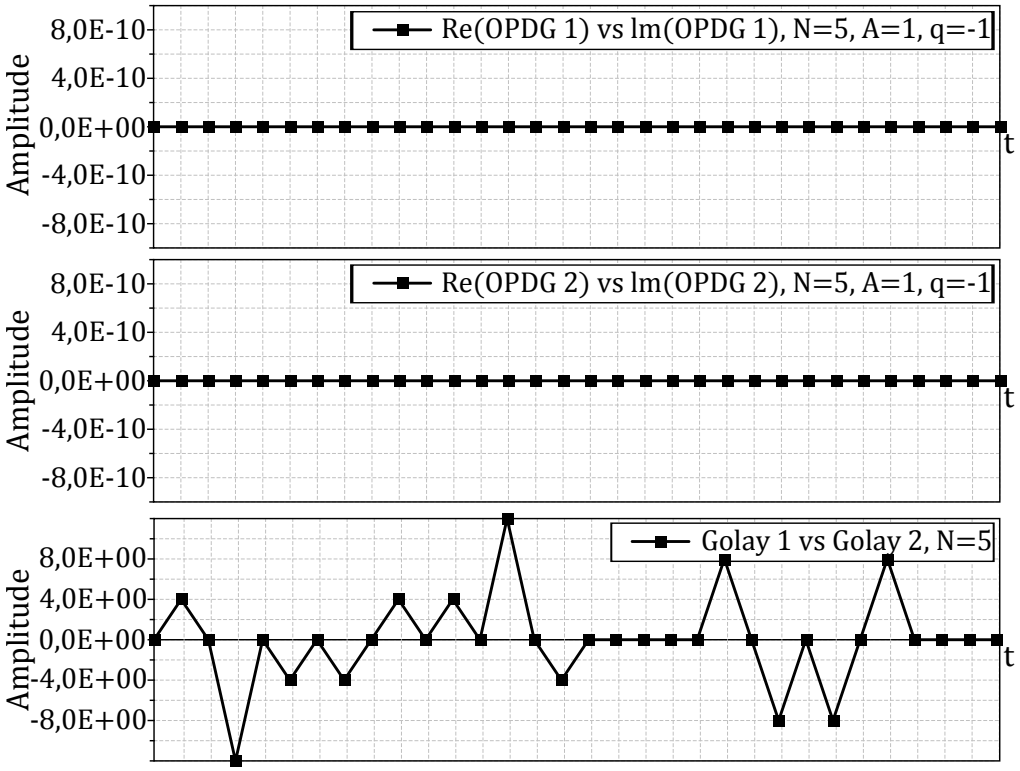


Figure 5: Periodic crosscorrelation amplitude vs. code index t, for length 2^5.

4 Conclusions

This letter proposes a novel codec that generates perfect sequences, which are based on Golay codes, through an IDFT. These sequences are compared with the original Golay codes, by checking through both crosscorrelation and autocorrelation functions. These new perfect sequences, with an autocorrelation proportional to a Dirac pulse, are theoretically immune to multi-path interference. Their real and imaginary parts are also orthogonal, with a constant zero crosscorrelation value between them. These properties indicate a better behavior of the OPDG codes, when compared with the original Golay codes.

References

[1] Donato, P. G., Funes, M. A., Hadad, M. N., and Carrica, D. O.: 'Optimised golay correlator', *Electron. Lett.*, 2009, **45**, (7), pp. 380-381

[2] Fan, P. Z., Darnell, M., and Honary, B.: 'Crosscorrelations of Frank sequences and Chu sequences', *Electron. Lett.*, 1994, **30**, (6), pp. 477-478

[3] Gold, R.: 'Optimal binary sequences for spread spectrum multiplexing', *IEEE Trans. Inf. Theor.*, 2006, **13**, (4), pp. 619-621

[4] Sarwate, D. V. and Pursley, M. B.: 'Crosscorrelation properties of pseudorandom and related sequences', *Proc. IEEE*, 1980, **68**, (5), pp. 593-619

[5] Zhou, Z., Tang, X., and Peng, D.: 'New optimal quadriphase zero correlation zone sequence sets with mismatched filtering', *IEEE Signal Processing Lett.*, 2009, **16**, (7), pp. 636-639

[6] Pereira, J. S. and Silva, H. J. A.: 'Optimal design of perfect DFT sequences', *PHYCOM*, 2013, **7**, pp. 92-104

[7] Pereira, J. S. and Silva, H. J. A.: 'Codificador e descodificador eletrónico de sinais ortogonais e perfeitos', *Portuguese Patent 106755 (20131000007826), Instituto Nacional da Propriedade Industrial (INPI)*, FCTUC, IT, IPL, 2014

Brussels view from the Museum of Music

Fabrication of biocompatible hydrogels from pine resin

by VIDHURA MAHENDRA

Centre for Rapid and Sustainable Product Development
Polytechnic Institute of Leiria
Corresponding author email: vidhura.mahendra@ipleiria.pt

Abstract This short review aims to look at some of the applications based on potential pine associated resin (rosin) composites for cell culture studies via hydrogel fabrication. Although hitherto there are only a few links to pine resin based hydrogel formation in the public domain, literature work based rosin incorporated drug delivery studies and its associated uses can be useful for extensive works on the cell interaction and viability. Rosin in such case may be optimised to afford similar characteristics hence applications.

1 Introduction

Pine resins are exudates ranging from the volatile terpenes to non-volatile material known as rosin (Figure 1). They are isolated by tapping the tree, approximately contains 70% rosin, 15% turpentine 15% debris and water [1]. At room temperature it is brittle and softens at higher temperatures. It is used in paper sizing, printing inks, surface coatings, adhesives and rubber additives together with some more advanced applications in biomedical and construction industry [2].

Figure 1: Blocks of rosin

2 Hydrogel fabrication from nature

The usual composition of hydrogels could be up to 99% water and as a result are similar to human tissues [3]. By tuning their shape, physical properties, chemical composition and infusing them with cells, biomedical engineers have successfully used hydrogels as three– dimensional molecular scaffolds that can be filled with cells, molecules for bodily injection or application in order to release drugs and stimulate tissue regeneration. Alginate hydrogels [4] have been studied as it is a biocompatible polysaccharide obtained from natural brown seaweed and its degradation kinetics can be tuned to suit drug molecules encapsulated in the gel hence delivery.

One of the most useful and naturally biocompatible polymers is cellulose and the research works related to them are encouraging. Peng et al [5] have reported on developing novel cellulose based hydrogels to overcome weak mechanical strength, poor biocompatibility and lack of antimicrobial activity which may induce skin allergy of the body in commercial diapers with a simple chemical cross-linking of quaternized cellulose (QC) and native cellulose in sodium hydroxide and urea aqueous solution. The prepared hydrogel was shown superabsorbent property, high mechanical strength, good biocompatibility and excellent antimicrobial efficacy against *Saccharomyces cerevisiae* (Figure 2). The resulting data encouraged the use of these hydrogels for hygienic application such as disposable diapers.

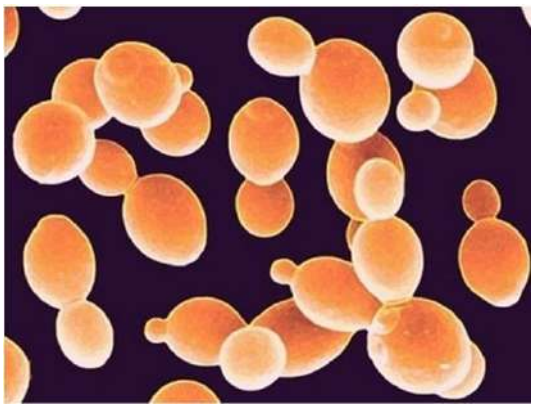


Figure 2: Saccromyces cerevisiae (unicellular fungi) Image credit: http://www.scientistlive.com/content/19429.

Kobayashi [6] describes the use of cellulose originat-

ing from bagasse wastes (Figure 3) from the food industry to fabricate hydrogel films with flexible and bioactive properties for tissue engineering.

Figure 3: Pile of baggase waste
Image credit:
http://www.endswasteandbioenergy.com/article/1290438/cuba-unveils-plan- 765mw-biomass-power

A natural plant polymer was regenerated from *Agave tequilana Weber* bagasse from Corralejo Penjamo, Guanajuato, Mexico. It was subsequently converted to lignocellulose. A phase inversion process with a new preparation technique was followed for cellulose hydrogel films. The hydrogel film preparation and characteristics were demonstrated from perspectives of bioactive applications with cytotoxicity of fibroblast cell cultivation on a scaffold film. Experimental evidence was established showing the resultant hydrogel films have exclusive properties displaying good mechanical and viscoelastic films even in their water-swollen condition. Hydrogel behaviours in cellulose structure and characteristics were clarified using several analytical methods for cell growth on the scaffold which was prepared to show different cellulose morphologies. Different effects of cellulose fibre nanostructures of the hydrogel films were described for their cytotoxicity for tissue engineering applications.

A new series of *in situ* forming antibacterial conductive degradable hydrogels using quaternized chitosan (QCS) grafted polyaniline (Figure 4) with oxidized dextran as cross- linker has been reported [7].

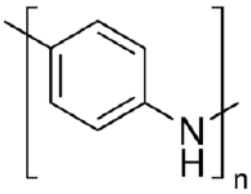
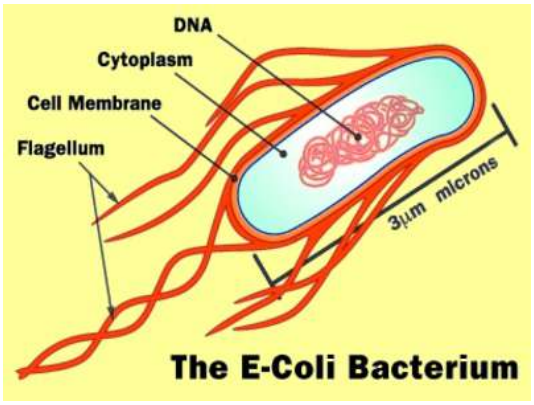
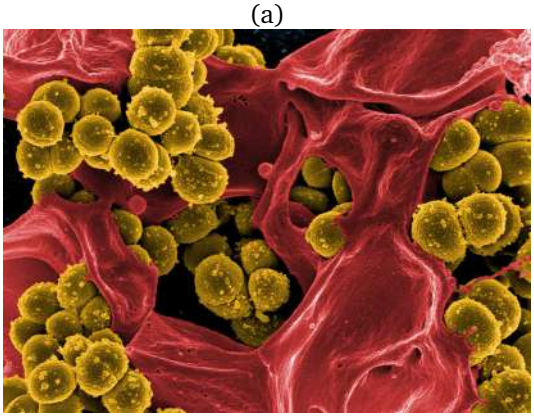


Figure 4: Chemical structure of polyaniline

The chemical structures, morphologies, electrochemical property, conductivity, swelling ratio, rheological property, *in vitro* biodegradation and gelation time of hydrogels were characterized. Injecting ability was verified by in vivo subcutaneous injection on a Sprague Dawley [8] rat. The antibacterial activity of the hydrogels was initially evaluated employing antibacterial assay using *Escherichia coli* and *Staphylococcus aureus in vitro* (Figure 5). The hydrogels containing polyaniline showed enhanced antibacterial activity compared to QCS hydrogel, especially for hydrogels with 3 wt% polyaniline showing 95 kill% and 90 kill% for *E. coli* and *S. aureus*, respectively. Compared with QCS hydrogel, the hydrogels with 3 wt% polyaniline still showed enhanced antibacterial activity for *E.coli in vivo*. The adipose-derived mesenchymal stem cells (ADMSCs) have been used to evaluate the cytotoxicity of the hydrogels, and hydrogels with polyaniline showed better cell compatibility than QCS hydrogel. The electroactive hydrogels could significantly enhance the proliferation of C2C12 myoblasts compared to QCS hydrogel. This work opens the way to fabricate in situ forming antibacterial and electroactive degradable hydrogels as a new class of bioactive scaffolds for tissue regeneration applications.



(a)



(b)

Figure 5: (a) Escherichia coli *and (b)* Staphylococcus aureus
Image credit (a):
http://www.nature-education.org/water-testing.html
Image credit (b):
This scanning electron micrograph shows the methicillin-resistant Staphylococcus aureus.
http://www.sci-news.com/medicine/science- antibiotics-methicillin-resistant-staphylococcus-aureus-01548.html

Franco et al [9] have described the anomalous

swelling behaviour of a scleroglucan (a water soluble nature-derived polysaccharide produced by fermentation of the filamentous fungus *Sclerotium rolfsii*) in borax hydrogel by different physico-chemical approaches and means of molecular dynamics simulations. The role of polymer combinations forming interpenetrated structures was explained in terms of specific properties which significantly differ from those of the constituent polymers thus allowing appropriate tailoring of the delivery rates. Finally the wide possibilities of applications of nano-gel structures which allow combination therapies for cancer treatment and the suitability for intracellular targeting have also been reported. The studies on polysaccharide hydrogels are still in progress and emphasize future researches to be more stimulated.

It is also noteworthy mentioning that natural biomaterials such as gelatine (derived either from animals or plants e.g. pectin or pectic polysaccharides) are directly involved in cell culture studies due to their biocompatibility. The chemical functionalities present in gelatin [10] (e.g. carboxylic acid, thiol, hydroxyl) allow for potential covalent modification of the gelatine methacrylated (GelMA) with growth factors or cytokines to further promote cell viability and function. Therefore, GelMA could potentially be tailored to different cell or tissue types or growth factor and drug delivery applications based on the specific type of gelatin precursor.

3 Rosin in biological applications

Along with the other naturally occurring materials previously discussed rosin has been studied as an application for drug delivery [11] obtained from *Pinus palustris* [12] (Figure 6), the long leaf pine. It has potential as pharmaceutical excipients [13], that is natural or synthetic substance formulated alongside the active ingredient of a medication in terms of biodegradability, ease of availability, matrix forming coating, microencapsulating and binding. The studies further reveal it had been found as an anti-inflammatory and antitumor activity. A semisolid preparation such as skin cream shows good homogeneity and spreading ability. Moreover consists of prominent properties for the sustained release drug system with most of the drug and dosage form.

Further study on rosin has been used to prepare spherical microcapsules by a method based on phase separation via solvent evaporation [14]. Rosin based polymer has been used as film coating materials; coated pellets were prepared using diclofenac sodium [15] as a model drug and sustained release of the drug was probed [16]. Rosin polymer has been used as the transdermal drug delivery system. Its combination with polyvinyl pyrrolidone and dibutyl phthalate (30% w/w) produces smooth film with improved elongation and tensile strength.

Figure 6: Longleaf pine (Pinus palustris) forest

Rosin has appropriate hydrophobic properties that can be utilized as matrix forming agent of water soluble drug such as dilitiazem hydrochloride [17] to prolong the release. The drug release followed first order kinetics and the Higuchi model, thus indicates that there was no erosion of the matrix and the tablet maintained its shape and surface area [18].

Moreover, rosin esters are reported to have good film forming properties and can be used for enteric coating and delayed release of drugs. Rosin and rosin-based polymers have drug delivery applications achieving sustained/controlled release profiles [19-20]. This further exemplifies the role of rosin as a barrier to migrate molecules in the medium.

Derivatives of rosin polymers (RD-1 and RD-2) had been synthesized in the laboratory and evaluated for physicochemical properties [21], polydispersity (Mw/Mn), molecular weight (Mw), and glass transition temperature (Tg). The derivatives of rosin have further been evaluated for pharmaceutical film coating by characterizing the release of a model drug (diclofenac sodium) from pellets coated with the derivatives. The studies have revealed that pellet film coating could be achieved without agglomeration of the pellets within a reasonable operation time and drug release was sustained up to 10 hours with the two rosin derivatives. These results have suggested the application of rosin derivatives (RD-1 and RD-2) for film coating.

In vitro tests related to rosin have been studied [22] to determine its biochemical and physical compatibilities. Free films of rosin (2 cm $\times$ 1 cm $\times$ 0.4 mm, 120 mg) were subjected to *in vitro* degradation by placing them in 10.0

mL of 0.2M phosphate buffered saline (PBS) (pH 7.4, 37 °C) and maintained on a rotating container [23]. The PBS was changed every 8 hours for the first day, every day for the first week and weekly thereafter to keep the pH relatively constant. 15 Films were withdrawn at intervals of 30, 60, and 90 days, washed with distilled water, dried and subjected to analysis. The films have been subcutaneously implanted on the backs of male Wistar [24] rats (200-300g) to monitor the *in vivo* degradation. Anaesthesia was induced by intraperitoneal injection of a mixture of ketamine HCl (85 mg/kg bodyweight) and xylazine (12 mg/kg body weight). Tetracycline, 10 mg/kg dose, was given at the time of surgery. An incision (2.5 cm) was inflicted laterally about the mid-portion of the back. Subcutaneous pockets were formed around each incision, free film was inserted, and the wounds have been sealed by intermittent nylon stitches at 0.5 cm apart. Films were explanted at 30, 60, and 90 days for analysis [25-26]. In these studies the authors have broadly justify the biocompatibility of rosin as a material for exclusive use in living organism.

Satturwar et al [27] have studied rosin, for its degradability and compatibility in and with the physiological environment with the aforementioned methodologies and revealed rosin has shown faster degradation *in vivo* as compared with *in vitro* studies. Subsequent placement in PBS, the rosin films showed MW loss of 14.7%, with the films being recovered at the end of 90 days. After *in vivo* implantation in rats, the free films showed MW loss of 60% at around day 75 and complete loss at the end of 90 days. Bulk degradation is evident both *in vitro* and *in vivo*. Although rosin degrades over a period of 2 to 3 months, it provides good compatibility compared with Poly (DL-lactic-co-glycolic acid) (PLGA) to the extent investigated in the paper. This finding presumably will lead to new applications of rosin in the field of drug delivery.

Nande et al have discussed the derivatives of rosin synthesized by a reaction with polyethylene glycol 200 and maleic anhydride proved suitable for sustaining drug release from matrix tablets and pellets [28]. Furthermore polymerised rosin films containing hydrophobic plasticisers showed excellent potential as coating materials for the preparation of sustained release dosage forms have been reported [29].

In a slightly different approach to what we have discussed so far, Kaith et al [30] have recently reported on reducing the gum rosin [31] from its rosin acid to alcohol form via a typical reducing agent, sodium borohydride and cross-linked subsequently with the addition of an acrylamide to afford its co-polymer as gum rosin-acrylamide (GrA-cl-poly(AAm) hydrogel, an application for removal of malachite green dye from waste water. This reaction resembles to the reaction of gelatine with acrylic anhydride followed by the cross-linking to afford gelatine methacrylamide (GelMA), a hydrogel which is widely used in cell culture studies as mentioned before. The paper has further explained the versatility of rosin in terms of modifying the structure to adopt a useful route in creating a hydrogel. Most importantly it also suggests the rosin acids collectively have no influence on reduction preferences to its alcohol form enabling to fabricate hydrogels as the final product.

4 Conclusion

In this short review we have looked into the ways of incorporating rosin as a useful precursor for potential biological studies similar to many other natural materials and its advancement. Number of researches carried out on rosin as a precursor for drug transport in biomedical applications and has been shown as a useful insulator for prolong released drugs *in vivo* with no influence in the overall interaction *per se*. Furthermore, it has also been studied for structural modifications especially in the hydrogel fabrications, where the rosin had undergone typical reduction reaction followed by polymerisation to afford the co-polymer. These studies have also proved that rosin withstands to structural modifications with its functional groups acting as the site of interest without the distortion of its overall fused ring system. These developments have encouraged for potential hydrogel fabrication for cell studies *in vitro* and *in vivo*.

Acknowledgement

We would like to acknowledge the Portuguese Foundation for Science and Technology (FCT — UID/ Multi/04044/2013), for the funding of the research work.

References

[1] Zhang J, Rosin-based chemicals and polymers, Smithers Rapra, 2012

[2] H F Enos, Current and potential use of Rosin, presented at Paper and Textile Chemistry Division Meeting, March 23, 1977, p.75.5 B. E. Avla and S. Pekkala, Journal of the American Chemical Society

[3] New method for synthesizing a biocompatible hydrogel could speed up research and development of several promising applications in tissue engineering http://tinyurl.com/zvj5xg2

[4] Alexander D. Augst, Hyun Joon Kong, David J. Mooney, Alginate Hydrogels as Biomaterials, Volume 6, Issue 8, August 7, 2006 , Pages 623–633, DOI: 10.1002/mabi.200600069

[5] Na Peng, Yanfeng Wang, Qif Ye, Lei Liang, Yuxing An, Qiwei Li, Chunyu Chang Biocompatible cellulose-based superabsorbent hydrogels with antimicrobial activity, Carbohydr. Polym, 2016 Feb 10; 137:59-64

[6] Takaomi Kobayashi , Chapter 1 – Fabrication of Cellulose Hydrogels and Characterization of Their Bio-

compatible Films, Studies in Natural Products Chemistry 45:1-5, Jan 2015

[7] Xin Zhao, Peng Li, Baolin Guo, Peter X. Ma, Antibacterial and conductive injectable hydrogels based on quaternized chitosan-graft-polyaniline/oxidized dextran for tissue engineering, Acta Biomater. 2015 Oct; 26:236-248

[8]Info from http://www.criver.com/products-services/basic-research/find-a-model/sprague-dawley-rat

[9] Franco Alhaique, Maria Antonietta Casadei, Claudia Cencetti, Tommasina Coviello, Chiara Di Meo, Pietro Matricardi, Elita Montanari, Settimio Pacelli, Patrizia Paolicelli, From macro to nano polysaccharide hydrogels: An opportunity for the delivery of drugs, J, Drug Delivery Science and Technology, 32, 88-99, 1 Apr 2016

[10] Jason W. Nichol, Sandeep T. Koshy, Hojae Bae, Chang M. Hwang, Seda Yamanlar, Ali Khademhosseini, Cell-laden microengineered gelatin methacrylate hydrogels, Biomaterials 31 (2010) 5536-5544

[11] Om Prakash Pal, Rishabha Malviya, Vipin Bansal, Pramod Kumar Sharma, ROSIN AN IMPORTANT POLYMER FOR DRUG DELIVERY: A SHORT REVIEW, International Journal of Pharmaceutical Sciences Review and Research, Volume 3, Issue 1, July – August 2010; Article 007 ISSN 0976 – 044X, pp 3-37

[12] Boyer, W. D. and J. B. White, Natural Regeneration of Longleaf pine, pp 99-113 in Proc. of the Symposium on the management of Longleaf Pine, Farrar RM (ed.) General Technical report 50–75

[13] Bhattacharyya, Lokesh; Schuber, Stefan; Sheehan, Catherine; William, Roger (2006). "Excipients: Background/Introduction". In Katdare, Ashok; Chaubal, Mahesh. Excipient Development for Pharmaceutical, Biotechnology, and Drug Delivery Systems. CRC Press.

[14] Bohme K, Buprenorphine in a transdermal therapeutic system–a new option, Clin Rheumatol, 21, 2002, Suppl 1: S13-16.

[15] Small RE, Diclofenac sodium, Clin. Pharm. 1989 Aug;8(8):545-58

[16] Pathak YV, Dorle AK, Rosin and rosin derivatives as hydrophobic matrix materials for controlled release of drugs, Drug Des Delivery, 6, 1990, 223-227.

[17] Info from http://www.rxlist.com/cardizem-cd-drug.htm

[18] Prashant MS, Suniket VF, Avinash KD, Evaluation of polymerized rosin for the formulation and development of transdermal drug delivery system: A Technical Note, AAPS PharmSciTech, 6(4), 2005, E649- E654.

[19] Pathak YV, Shinghatgiri M, Dorle AK, *in vivo* performance of pentastergum coated aspirin microcapsules, J Microencapsul, 4, 1987, 107Y110.

[20] Sheorey DS, Dorle AK, Release kinetics & drugs from rosin-glycerol ester microcapsules prepared by solvent evaporation technique, J Microencapsul, 8, 1991, 243Y246.

[21] Lakshmana PS, Shirwaikar AA, Shirwaikar A, Kumar A, Formulation and evaluation of sustained release microspheres of rosin containing aceclofenac, 50(2), 2009, 51-62.

[22] Lu L, Garcia CA, Mikos AG. *in vitro* degradation of thin poly (DL-lactic-co-glycolic acid) films. J Biomed Mater Res. 1999; 46: 236-244.

[23] Suggs LJ, Krishnan RS, Garcia CA, Peter SJ, Anderson JM, Mikos AG. *in vitro* and *in vivo* degradation of poly (propylene fumarate-co-ethylene glycol) hydrogels. J Biomed Mater Res. 1998; 42: 312-320.

[24] Info from http://www.criver.com/products-services/basic-research/find-a-model/wistar-rat

[25] Schakenraad JM, Nieuwenhuis P, Molenaar I, Helder J, Dijkstra PJ, Feijen J. *in vivo* and *in vitro* degradation of glycine/DL-lactic acid copolymers. J Biomed Mater Res. 1989; 23: 1271-1288.

[26] Gogolewski S, Jovanovic M, Perren SM, Dillon JG, Hughes MK. Tissue response and *in vivo* degradation of selected polyhydroxyacids: polylactides (PLA), poly (3-hydroxybutyrate) (PHB) and poly(3- hydroxybutyrate-cp-3-hydroxyvalerate) (PHB/VA). J Biomed Mater Res. 1993; 27: 1135-1148.

[27] Prashant M. Satturwar, 1 Suniket V. Fulzele, 1 and Avinash K. Dorle 1, Biodegradation and *in vivo* Biocompatibility of Rosin: a Natural Film-Forming Polymer, AAPS PharmSciTech 2003; 4 (4) Article 55 (http://www.aapspharmscitech.org), pp 1-6

[28] Nande, V.S.; Barabde, U.V.; Morkhade, D.M.; Patil, A.T.; Joshi, S.B. Synthesis and characterization of PEGylated derivatives of rosin for sustained drug delivery. Reactive Funct. Polym. 2006, 66, 1373-1383.

[29] Fulzele, S.V.; Satturwar, P.M.; Dorle, A.K. Polymerized rosin: novel film forming polymer for drug delivery. Int. J. Pharm. 2002, 249, 175-184.

[30] B. S. Kaith, Rajeev Jindal and Rachna Sharma, Synthesis of a Gum rosin alcohol-poly(acrylamide) based adsorbent and its application in removal of malachite green dye from waste water, RSC Adv., 2015, 5, 43092–43104, DOI: 10.1039/c5ra04256a

[31] Y. Zheng, K. Yao, J. Lee, D. Chandler, J. Wang, C. Wang, F. Chu and C. Tang, Macromolecules, 2010, 43, 5922–5924.

On the structure of a triangulation

by NELSON MARTINS-FERREIRA

Centre for Rapid and Sustainable Product Development
Polytechnic Institute of Leiria
martins.ferreira@ipleiria.pt

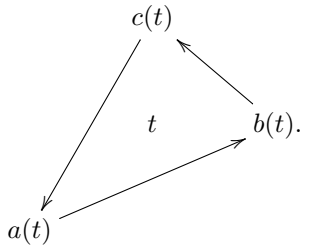
Abstract We characterize the notion of a triangulation, in which every vertex has a star-neighbourhood, in terms of an internal categorical structure. This structure is important since it allows to have more efficient and robust algorithms that can be used in additive manufacturing.

0.1 Introduction

The structure of a triangulation generalizes the one of a directed graph. If $\mathbb{C}$ is a category then an internal directed graph in $\mathbb{C}$ consists of a pair of objects (the object of vertices and the object of edges) together with two parallel morphisms between them, called the domain and codomain morphisms. A triangulation, in our sense, consists of two objects and three parallel morphisms between them, as displayed

$$T \overset{\overset{a}{\overset{b}{\underset{c}{\rightrightarrows}}}}{\rightrightarrows} V, \tag{1}$$

with an element $t \in T$, say in the category of sets and maps, being interpreted as a triangle in the following manner



In practice we are concerned with triangulated surfaces. Suppose we are given a triangulation

$$T \overset{\overset{a}{\overset{b}{\underset{c}{\rightrightarrows}}}}{\rightrightarrows} \mathbb{R}^3, \tag{2}$$

in the category `Sub64`, whose objects are all sets and the morphisms are those maps whose domain is either infinite (in which case they are required to be bijections) or else have a cardinality not greater than 2^{64}. This particular category can be used as a model for computational systems such as `Matlab` or `Julia`.

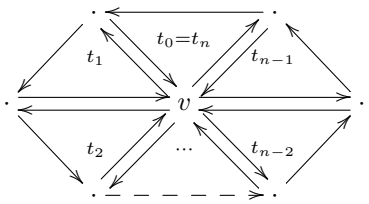
0.2 The problem

The following problem arises in the area of additive manufacturing [1] which is concerned with the production of 3-D real world objects using the so-called layer by layer fabrication.

How can we distinguish the triangulations (2) which are enclosing a physical real object in the 3-D euclidean space from the ones who don't?

The answer is fairly simple and well-known: each vertex $v \in V$ should admit a star-neighbourhood. Or, in other words, the set of all triangles in T, that are incident with the vertex $v \in V$, admits a cyclic order that is compatible with the adjacency of the edges, as illustrated.

(3)



This well-known characterization, however, has some draw-backs with respect to computational implementations and algorithms that operate on it [1]. Hence the need for finding an alternative solution to this problem. We have thus found an alternative which is equivalent and more appropriate to our needs.

First we replace $\mathbb{R}^3$ with the Cayley algebra of quaternions $\mathbb{H}$, see e.g. [3], this is only a technical issue and in practice even the algebra of octonions is of great use since it allows to take into account other physical properties such as color, type of material, density, etc.

Secondly, instead of triangulations of the form

$$T \overset{\overset{a}{\overset{b}{\underset{c}{\rightrightarrows}}}}{\rightrightarrows} \mathbb{H} \tag{4}$$

in `Sub64`, we consider the structures of the form

$$\theta,\varphi \circlearrowright A \xrightarrow{\ g\ } \mathbb{H} \tag{5}$$

such that

$$\theta^3 = 1_A \tag{6}$$

$$\theta^2 = \varphi\theta\varphi \tag{7}$$

$$g\varphi = g \tag{8}$$

and we can show that the *star-neighbourhood* property displayed in (3) is equivalent to the requirement that φ is an isomorphism.

0.3 The solution

We are now going to give a quick explanation on how to transform (4) into (5) and vice-versa.

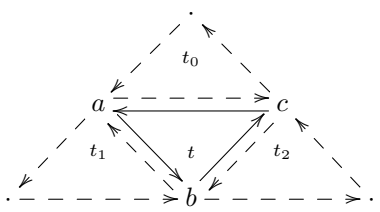
Having a structure such as (4) with the star-neighbourhood property for each of its vertices, in order to obtain a structure such as (5), we define:

$$\begin{aligned} A &= T \times \{0,1,2\} \\ \theta(t,i) &= (t, i+1 \mod 3) \\ g(t,i) &= \begin{cases} a(t) & \text{if } i=0 \\ b(t) & \text{if } i=1 \\ c(t) & \text{if } i=2 \end{cases} \end{aligned}$$

and

$$\varphi(t,i) = (t_i, j(t,i))$$

with t_i, $i = 0,1,2$ as illustrated



and $j(t,i)$ given by

$$j(t,0) = \begin{cases} 0 & \text{if } a(t_0) = a(t) \\ 1 & \text{if } b(t_0) = a(t) \\ 2 & \text{if } c(t_0) = a(t) \end{cases}$$

$$j(t,1) = \begin{cases} 0 & \text{if } a(t_1) = b(t) \\ 1 & \text{if } b(t_1) = b(t) \\ 2 & \text{if } c(t_1) = b(t) \end{cases}$$

$$j(t,2) = \begin{cases} 0 & \text{if } a(t_2) = c(t) \\ 1 & \text{if } b(t_2) = c(t) \\ 2 & \text{if } c(t_2) = c(t) \end{cases}$$

Clearly, each vertex has a star-neighbourhood, if and only if φ is a bijection. Note that we are working on the category `Sub64`, which in particular has the property that every regular epimorphism is a split epimorphism. Also note that, in order to construct φ it is sufficient that each triangle in (4) to have three adjacent triangles, one for each edge, which is a weaker condition that the star-neighbourhood property.

Conversely, if having a structure such as (5) with its three conditions, then we define a triangulation as follows. The triangles are obtained by identifying the orbits of θ, via the coequalizer $p = coeq(1_A, \theta)\colon A \to T$ (note that this particular coequalizer exists in `Subd64`, even though its kernel relation may fail to exist); by identifying the orbits of φ, say with $q = coeq(1_A, \varphi)\colon A \to V$, we obtain unique labels for the vertices in the triangulation. The complete structure, with $a = qs$, $b = q\theta s$ and $c = q\theta^2 s$ (where s is any section for p) is displayed as

$$T \underset{c}{\overset{a}{\underset{}{\overset{}{\underset{}{\rightrightarrows}}}}} V \xrightarrow{m} \mathbb{H}$$

with m the unique map such that $g = mq$.

0.4 Conclusion

The structure (A, θ, φ, g), with conditions (6)–(8) and the extra requirement that $varphi$ is an isomorphism, when internal to the category `Sub64`, is equivalent to the structure of a triangulation (T, V, a, b, c) satisfying the star-neighbourhood property. The advantage of this new structure is that it allows the development and implementation of several new algorithms and other geometrical constructions in a more robust and efficient way. An important example is the slicing algorithm which decomposes the triangulation into several disjoint contour levels and permits it fabrication with a layer by layer technique.

As a final remark we observe that a somewhat different but related problem is the one of characterizing those triangulations (1) that can appear as the multiplicative structure of an internal groupoid, see [5] for more details: a triangulation such as (T, V, a, b, c) displayed in (1) is the multiplicative structure of an internal groupoid if and only if the two directed graphs (T, V, a, b) and (T, V, b, c) centralize each other and moreover the pushout of the span (a, c) is a pullback square.

This work was presented in the conference [6]

References

[1] M. B. Gaspar and N. Martins-Ferreira, *Robust STL processing for extrusion-based manufacturing*, Innovative Developments in Virtual and Physical Prototyping, 2012 , 273–278.

[2] N. Alves, P. Bártolo, N. Martins-Ferreira, M. B. Gaspar and A. Mateus, *BioFab toolbox – Software tools for biofabrication*, High Value Manufacturing: Advanced Research in Virtual and Rapid Prototyping, 2013, 483–487.

[3] Ian R. Porteous, *Topological Geometry*, Van Nostrand Reinhold and Company, 1969.

[4] Miguel Belbut Gaspar and Nelson Martins-Ferreira. A procedure for computing the symmetric difference of regions defined by polygonal curves. *J. Symbolic Comput.*, 61/62:53–65, 2014.

[5] N. Martins-Ferreira, *On the multiplicative structure of internal groupoids*, in preparation.

[6] N. Martins-Ferreira " On the multiplicative structure of internal categories and internal groupoids (Poster)", CatAlg2015, 3rd Workshop on Categorical Algebra: Recent developments and future perspectives, Gargnano, 26-30Apr2015, Italy