Journal of Plastic, Reconstructive & Aesthetic Surgery (2007) 60, 1110–1119

JPRAS
An International Journal of Surgical Reconstruction
www.JPRASurg.com

REVIEW

Hyaluronic acid: the scientific and clinical evidence

Richard D. Price [a,b,*], **MG Berry** [c], **Harshad A. Navsaria** [b]

[a] *Addenbrooke's Hospital, Cambridge, UK*
[b] *Centre for Cutaneous Research, Bart's and London School of Medicine and Dentistry, Queen Mary, University of London, 4 Newark Street, London E1 2AT, UK*
[c] *The Royal Prince Alfred Hospital, Sydney, NSW, Australia*

Received 6 June 2006; accepted 5 March 2007

KEYWORDS
Hyaluronic acid;
Tissue engineering;
Fillers

Summary Hyaluronic acid is a naturally occurring biopolymer whose molecular structure is highly conserved between mammalian species. First described in 1934, it has since been used across a wide variety of medical fields as diverse as neurosurgery and cutaneous wound healing. Presently it has reached prominence in cosmetic practice where it is now the injectable dermal filler of choice for most surgeons.

We used our experience of this technology with searches in the English language literature for the purpose of a systematic review. We present an overview, including the scientific evidence for its use in wound healing and, briefly, in other fields. We summarise the evidence for and against hyaluronic acid and provide a resumé of the current technologies available in fields such as skin regeneration and wound healing, in addition to cosmetic surgery. This overview is not intended to teach the reader about the various formulations currently on the market or how to use these materials clinically — rather to provide a solid scientific background enabling the reader to understand the attributes (and otherwise) of the material. We hope to allow clinicians to assess the evidence for a material now in common use in order that they may be fully aware of its properties.

Tissue engineering is on the cusp of reality in medicine. It has been 30 years since the first description of human keratinocyte culture, 25 years since the first dermal analogue and in the interim numerous cytokines and inflammatory mediators that modulate wound healing have been discovered (review,

* Corresponding author. Address: Addenbrooke Hospital, Cambridge, UK. Tel.: +44 161 291 6648.
E-mail address: rdp@rick-price.co.uk (R.D. Price).

1748-6815/$ - see front matter © 2007 Published by Elsevier Ltd on behalf of British Association of Plastic, Reconstructive and Aesthetic Surgeons.
doi:10.1016/j.bjps.2007.03.005

Martin 1997[1]). These discoveries have fuelled the development of materials for a variety of applications and readers will be familiar with examples such as Integra® and Apligraf® (Table 1). Bioengineered materials have also been trialled in fields as diverse as orthopaedic, spinal, and tracheal surgery, although not yet in routine use in any of these.

Since collagen forms the largest single component of the extracellular matrix, it is understandable that this was the focus for early investigators. The first stable collagen matrix was developed by Bell in the 1980s,[2] a precursor of the commercial product Integra®, now commonly used as a scaffold for dermal regeneration. Being such an abundant material in nature, the simplest way of collagen production is extraction from animal, primarily bovine, tissues. Importantly, collagen chemistry is species-specific, so that human, bovine and porcine collagens are biologically different: for injection, allergy testing may be required, explaining its recent decline as an injectable filler.

Hyaluronic acid (HA) forms a smaller part of the extracellular matrix (ECM) but has the significant advantage of structural conservation regardless of the source and is therefore nonallergenic. It is also relatively rare within the field of tissue engineering as its degradation products may be able to modulate wound healing. This has led to a wealth of research allowing the use of HA in clinical applications as diverse as dermal scaffolds, cartilage defects, glial cell culture and regeneration and even for spermatic motility assessment.

Biology of hyaluronic acid

First discovered in the vitreous humour of the eye in 1934[3] and subsequently synthesised in vitro in 1964,[4] HA consists of a basic unit of two sugars, glucuronic acid and *N*-acetylglucosamine, polymerised into large macromolecules of over 30 000 repeating units. It is therefore one of the largest components of the ECM, whose structure appears identical throughout phyla and species as diverse as *Pseudomonas* slime, *Ascaris* worms and mammals such as the rat, rabbit and human. Equally, its presence has been documented in tissues as diverse as the skin, aorta, cartilage and brain.

The molecule is readily soluble in water, producing a gel (Fig. 1) that behaves as a lubricant as well as adsorbing water, lending it hygroscopic and homeostatic properties. The high solubility of hyaluronic acid has proven to be problematic in the development of polymers for tissue engineering: a sheet placed in even a small amount of water liquefies. This may be beneficial in some applications, for example orthopaedic surgery (see below), but more structural stability is required for tissue engineering and chemical stabilisation is necessary. The viscosity of the gel produced appears to be dependent upon a number of properties including the length of the chains (and by extension the degree of entanglement[5]), cross-linking,[6] pH and chemical modification.[7] Interestingly, the absolute concentration appears to be much less important in this regard.

Alcohol esterification is the usual method of stabilisation, generally benzyl alcohol (see Campoccia et al.[8]). In essence, this cross-links the polymer to a variable degree leading to molecules with differing solubilities. Cross-linking and esterification are thought to prevent water ingress, reducing its solubility. Additionally, esterification prevents fibroblast binding, so reducing cellular degradation; the 75% esterified material is degraded over 7–14 days, whilst the 95% product may be present in tissue sections after up to 2 months.

Biosynthesis and degradation

HA may be produced from a number of sources but the two most common are extraction from rooster combs, and recombinant production using *Streptococcus* bacterium, each product having slightly different rheological properties.[9] In animals HA is formed at the cell surface of fibroblasts by extrusion into the ECM in close association with a dedicated receptor, CD-44 (see below). Fibroblasts also elaborate hyaluronidase, the degradation enzyme, and are able to internalise both HA and, importantly, its breakdown products.[10]

Enzymatic degradation cleaves the HA macromolecule into smaller polymers, each comprised of variable lengths of dimeric chains, many of which appear to modulate wound healing – although studies have indicated that most of the effects attributed to the molecule are applicable to only a few products. Purely for convenience, the fragments are now described as short- or long-chain.

The rôle of hyaluronic acid in wound healing and scar formation

Extracellular matrix regeneration

Collagen deposition by fibroblasts is one of the key factors in reconstituting supporting matrix at sites of scar formation and it is the nature of this deposition that largely determines scar quality. Long-chain HA appears to stimulate fibroblast proliferation, both in gingiva[11] and *in vitro*,[12,13] although it prevents adhesions in peripheral nerves,[14] and decreases the total scar collagen production by adult (but not foetal – see below) dermal fibroblasts.[15] There is also evidence that ECM remodelling following HA application is enhanced and collagen deposition more ordered.[16–18] Paradoxically, hyaluronidase (which would be expected to increase tissue HA fragments) causes increased scarring,[19] whilst persistently raised levels of HA decrease fibroblast contraction.[20] Further, fibroblast production of HA may be affected by a number of growth factors[21] and HA degradation products are pro-angiogenic, the effect being limited to fragments of between four and 25 disaccharides in length.[22]

Foetal wound healing has been extensively studied in the search for scarless healing. The raised levels of HA found in adult wounds fall after the initial proliferative phase, but remain elevated in foetal wounds throughout maturation[23]; the latter not by increased production, rather by decreased turnover. HA appears not to be degraded in the foetal environment possibly as a result of the rheological effects (see above). When foetal forelimbs are cultured *in vitro* and wounds created, application of exogenous HA decreases scarring.[24] Concurrently, early foetal wounds demonstrate less CD-44 and receptor for hyaluronic acid-mediated motility (RHAMM) expression than adult wounds, which may partly explain the increase in scarring seen as gestation progresses.[25]